The effect of temperature and soil water on *Fusarium* seedling blight of winter wheat and its effective control by fungicide seed treatments

Thesis

THE EFFECT OF TEMPERATURE AND SOIL WATER ON *FUSARIUM* SEEDLING BLIGHT OF WINTER WHEAT AND ITS EFFECTIVE CONTROL BY FUNGICIDE SEED TREATMENTS

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Harper Adams University College in collaboration with Crompton Europe Limited.

For Claire

Let Forever Be.....

Declaration

This thesis was composed by the author and is a record of work carried out by him on an original line of research. All sources of information are shown in the texts and listed in the references: all help given by others is indicated in the acknowledgements.

None of this work has been presented in any previous application for any degree or qualification.

Signed.....(Ian M. Haigh)

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ABSTRACT

Seed-borne *Microdochium nivale* adversely affected seed imbibition and germination to a greater extent at low temperatures (5 °C) and low soil water potentials (<-1 MPa) than at high temperatures (≥10 °C) and high soil water potentials (>-1 MPa). Post-emergent seedling blight severity was related to rate of seedling emergence, and adversely affected first leaf lengths.

In vitro base temperatures for growth of *M. nivale* var. *majus* isolates were lower than for *M. nivale* var. *nivale* isolates, however var. *majus* isolates had a faster growth rate. *Microdochium nivale* DNA from seed-borne infection, in seeds and seedlings, was not substantially increased at growth stage (GS) 01 or GS 10 at low temperatures or reduced soil water contents.

Timing, duration and harshness of freezing significantly affected seedling blight severity from seed-borne *M. nivale* infection. Pre-emergent exposure to -5 °C more severely reduced final emergence than exposure to 0 °C. Post-emergent freezing had less severe effects on seedling blight severity. Carboxin + thiram seed treatment increased final emergence and reduced seedling blight severity under conditions involving exposure to freezing temperatures.

Seed-borne *M. nivale* caused foot rot disease, even in the absence of seedling blight symptoms. Seedling blight severity adversely affected subsequent plant growth and was related to the extent of stem colonisation. Carboxin + thiram seed treatment reduced foot rot disease incidence and stem colonisation from seed-borne *M. nivale* in pot trials and increased plant productivity.

Soil water content and soil temperature between drilling and 30 days post-drilling, had significant effects on plant emergence from *M. nivale* diseased and pathogen-free seeds in field trials. Poor emergence occurred from seedlots with high seed-borne *M. nivale* infection in all three years of field trials and also in one year for a seedlot with low seed-borne *M. nivale* infection and low seed vigour.

Fungicide seed treatments provided robust control of *M. nivale* seedling blight under a range of seedbed conditions. Soil water content and soil temperature at drilling and up to 30 days after drilling were important determinants of emergence from treated seeds. Carboxin + thiram was the most effective seed treatment under all seed bed conditions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

LIST OF APPENDICES

CHAPTER 1

Introduction

INTRODUCTION

In the UK, a complex of fungal pathogens including *Fusarium avenaecum*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium poae* and *Microdochium nivale* are able to infect cereal plants causing the diseases: seedling blight, foot rot and ear blight. Of these species, *M. nivale* and *F. culmorum* are considered to be the predominant pathogens causing all three diseases in the UK whilst *F. avenaecum* and *F. poae* are predominantly associated with ear blight. The incidence of *F. graminearum* in UK cereal crops is low due to the fact that the temperate maritime climate of the UK is not conducive to a pathogen more frequently associated with hotter continental climates. The work described here concentrated on seedling blight caused by *M. nivale*, which is believed to be the predominant seedling blight pathogen in the UK. However the substantial research conducted into seedling blight caused by *F. culmorum* and *F. graminearum* is also reviewed where relevant.

Considerable research has been conducted into seedling blight of winter wheat. Numerous investigations in pot trials have demonstrated that *Microdochium nivale* seedling blight is most severe in cold dry soils. The use of seed treatments can increase final emergence and reduce disease severity compared to untreated seed from *M. nivale* infected seedlots. More recently, attempts have been made to understand the somewhat complex interactions that determine seedling blight severity or seedling disease escape. For example, Hare *et al.* (1995) demonstrated that rate of seedling emergence was closely related to final emergence from naturally infected *M. nivale* seedlots in controlled environment trials. However, many of the intricacies of the seed, seedling and *M. nivale* interaction remain undiscovered.

The main limitation of the work to date is that it was conducted under constant soil conditions, which do not occur under field conditions. Therefore, given current agricultural trends towards reduced seed rates and sowing of untreated seed, it was considered appropriate to conduct an investigation into the effect of soil water content and temperature on seedling blight of winter wheat and its effective control by fungicide seed treatments.

CHAPTER 2

Literature Review

LITERATURE REVIEW

Classification of *Microdochium nivale*

Microdochium nivale (Fr.) Samuels & Hallet was previously classified as *Fusarium nivale* and *Gerlachia nivalis* (see Table 1). There are two *M. nivale* sub-species, var. *majus* and var. *nivale*. Wollenweber (1931) first reported that *M. nivale* var. *majus* has larger conidia than *M. nivale* var. *nivale*. This was later confirmed by the work of Gerlach & Nirenberg (1982). Polymerase Chain Reaction (PCR) techniques are now considered more accurate to distinguish the sub-species, due to reduced variability of DNA compared with conidium width (Lees *et al.*, 1995).

Table 1. Classification of *Microdochium nivale*.

imperfect state (anamorph)	**perfect state (teleomorph)**
Microdochium nivale (Fr.) Samuels & Hallett	*Monographella nivalis* (Schaffnit) Muller
= *Fusarium nivale* (Fr.) Ces	= *Micronectriella nivalis* (Sch.) Booth
= *Gerlachia nivalis* (Ces.) Gams & Muller	= *Calonectria nivalis* Sch.
	= *Griphosphaeria nivalis* (Sch.) Muller & Arx.
	= *Calonectria graminicola* Wollenweber

Distribution of *Microdochium nivale*

Microdochium nivale is a widespread and important pathogen of cereals in cooler climates. In the UK, the important diseases caused by *M. nivale* are seedling blight, foot rot and ear blight. Numerous surveys of *M. nivale* occurrence in commercial crops have been conducted. In a survey in Scotland between 1970-1974, *M. nivale* was isolated from 42 % of 2657 diseased winter wheat seedlings and 4 % of 13823 seedlings showing no disease symptoms (Rennie *et al.*, 1983). However, no information was provided about the number of sites sampled. A survey of 375 winter wheat crops at growth stage (GS; Zadoks *et al.*, 1974) 73-75 in England and Wales in 1986 by Locke *et al.* (1987) more accurately determined the distribution of *M. nivale*. In this work, *M. nivale* was the predominant Fusarium pathogen on stems, foliage and ears. Possibly the most complete survey

undertaken was by Daamen *et al.* (1991) who sampled winter wheat crops in Holland over 12 years and seedlots over 14 years. In this study, *M. nivale* was frequently the most common pathogen isolated from seeds, seedlings, stem-bases, leaves and leaf sheaths.

All of the above investigations grouped the two *M. nivale* sub-species together. However, *M. nivale* var. *majus* may be more frequent in wheat and *M. nivale* var. *nivale* more common in oats. Evidence came initially from a one year study by Parry *et al.* (1995). Ninety three percent of 91 isolates from wheat grain samples and 70 % of 144 isolates from stem-bases of winter wheat in England and Scotland were var. *majus*. However, the sample size was limited to seven sites for grain samples and thirty sites for stem-base samples. Further work by Diamond & Cooke (1997) sampled a more extensive area. Of 71 isolates from cereal seeds harvested in 1994 and 152 isolates from seeds harvested in 1996 from around Ireland, almost all isolates from oats were var. *nivale*. Unfortunately, the number and distribution of crop samples was not provided. In subsequent work by Turner *et al.* (1999), var. *majus* was the principle *M. nivale* brown foot rot causal agent, determined by PCR, on winter wheat (cv. Beaver) at GS 37 in 1992-1993 at two sites in the UK. However, in field trials at three sites over three years, the incidence of *M. nivale* var. *majus* and var. *nivale* determined by PCR, was similar on winter wheat stem-bases at GS 12-31 and GS 32-45 (Turner *et al.*, 2002).

Differences in the two *M. nivale* sub-species maybe due to differences in pathogenicity. However, Maurin *et al.* (1995) recorded no difference in pathogenicity of *M. nivale* var. *majus* and var. *nivale* isolates to winter wheat (cv. Camp-Remy) seedlings. Simpson *et al.* (2000) reported that *M. nivale* var. *majus* had a weak selective advantage over var. *nivale* on wheat and oat seedlings, whilst *M. nivale* var. *nivale* showed a strong selective advantage over var. *majus* on rye seedlings. At 6 °C, inoculation of wheat seed with *M. nivale* var. *majus* spores reduced seedling emergence and produced more severe disease

symptoms than *M. nivale* var. *nivale* (Hare, 1997). Glynn (2002) confirmed Hare's (1997) findings and demonstrated that the same trend occurred for natural seed infection.

Microdochium nivale epidemiology

Microdochium nivale is found on all cereal parts in cool climates (Figure 1). Its dispersal through the crop is affected by weather conditions. From observations of wheat crops between 1961-1964, Millar & Colhoun (1969a) suggested wet summers encouraged rapid *M. nivale* spread through the crop canopy by air dissemination of ascospores from perithecia and conidia from stem lesions.

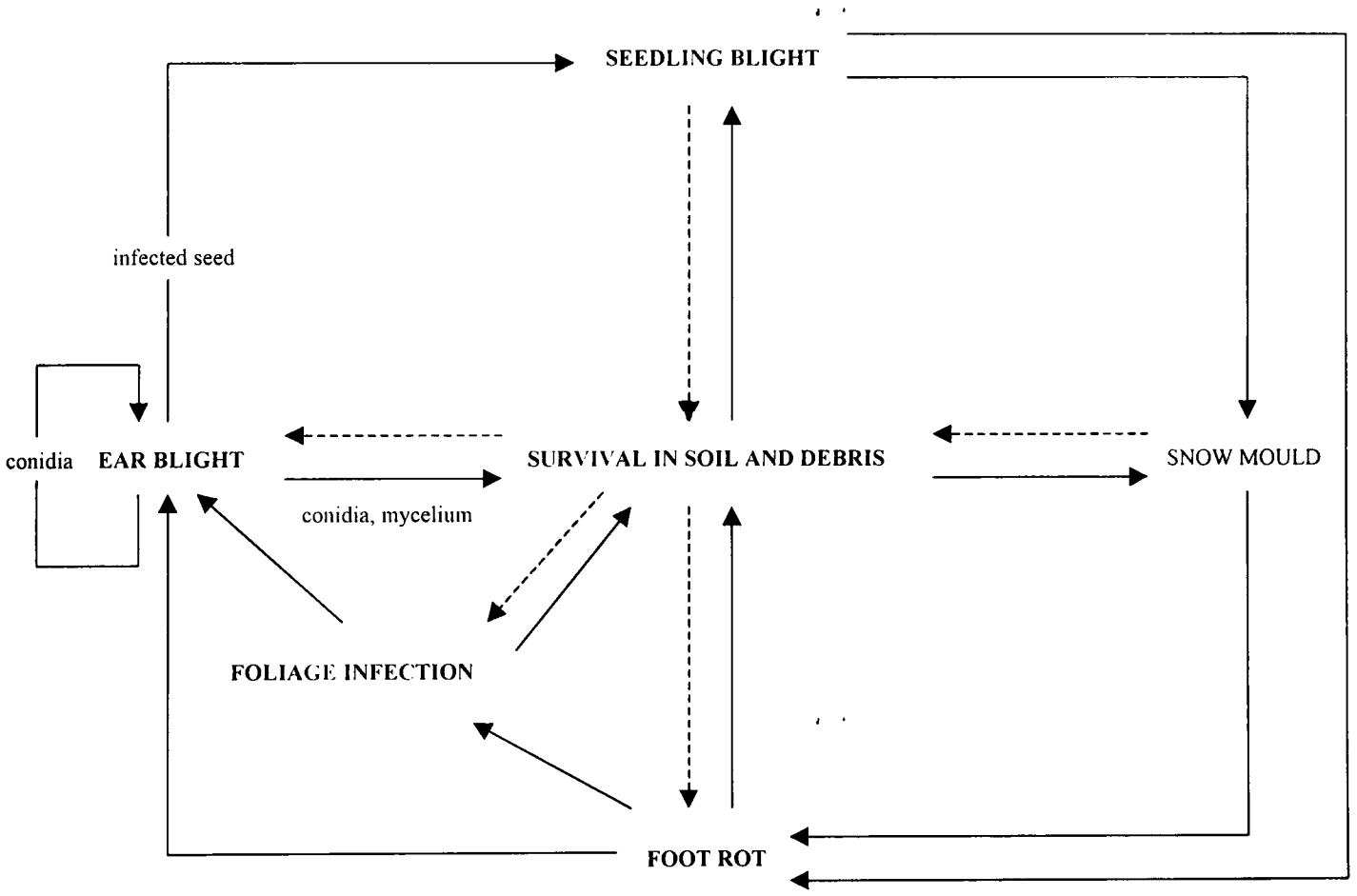


Figure 1. *Microdochium nivale* disease cycle on cereals (adapted from Cook (1981) and Parry *et al.* (1993)).

Jenkinson & Parry (1994) demonstrated splash dispersal of *Fusarium* spp. Conidia of *F. culmorum* and *F. avenaceum* from sporodochia on wheat stems were trapped on selective agar 100 and 90 cm away horizontally, and 60 and 45 cm away vertically respectively, after dispersal by simulated raindrops in still air. In a 1992 field trial using natural foot rot infection, Jenkinson (1994) found a significant correlation (R^2 = 0.73) between rainfall

amount day^{-1} in June and number of spores day^{-1} trapped in a Rotorod spore sampler 1 m above a winter wheat (cv. Longbow) crop. No significant relationship occurred for July rainfall and number of spores trapped. There was also no significant relationship between wind-speed and spore numbers over the crop, or spore numbers on stem-bases and spore numbers above the crop. Rossi *et al.* (2000) also carried out spore trapping within or above the wheat canopy over six years of field trials. Increased *F. avenaceum*, *F. culmorum*, *F. graminearum* and *M. nivale* conidia numbers were trapped with increasing number of rainy days from GS 51. Highest spore numbers occurred after rainfall had ceased. The distribution of rainfall was also important. Rainfall over two to three days increased conidia numbers compared to many isolated rain events. Prolonged intervals of dry days between rainfall reduced spore numbers.

There is limited evidence that internal colonisation of cereals by *Fusarium* pathogens may also contribute to disease during the season. There is however, conflicting information on how far up the plants *Fusarium* pathogens can colonise. In work by Snijders (1990), crown rot from *F. culmorum* inoculated on the roots and stem-bases of 12 winter wheat cultivars led to infection of the higher stem internodes but not the ears. In controlled environment investigations by Hutcheon & Jordan (1992), *F. avenaceum*, *F. culmorum*, *F. graminearum* and *M. nivale* were recovered from all internodes and the ear after inoculation of winter wheat (cv. Avalon) stem-bases at GS 21. *Microdochium nivale* was the most frequently isolated species from the ears. However, the experimental approach means splash dispersal may have been responsible. The controlled environment work of Clement & Parry (1998) was designed to prevent conidia splash dispersal. Soil-borne *F. culmorum*, *F. graminearum* and *M. nivale* colonised up to the fourth node of winter wheat (cv. Cadenza) but were not recovered from the fifth node or ears. Therefore, systemic colonisation from *Fusarium* soil-borne inoculum can cause disease on the stem but it appears that splash dispersal is required for ear infections.

The only evidence for *M. nivale* disease increasing in cereal crops during the season comes from investigations that isolated *M. nivale* from plant tissue. Rennie *et al.* (1983) surveyed 339 Scottish winter wheat crops between 1971 and 1974. *Microdochium nivale* was isolated from 5-10 % of seedlings, whilst July isolations yielded *M. nivale* from 15-25 % of adult plants. However, there was no mention as to which plant parts were sampled. Hare (1997) demonstrated that *M. nivale* inoculum was not uniform during the season from wheat plants growing from naturally infected untreated seeds. Disease incidence on stem-bases was greatest at GS 10-11, reduced at GS 30-31, increased again at GS 45 before declining at GS 75. At a second site, *M. nivale* isolation incidences remained fairly constant throughout the season. The reasons for this are unknown but it may be due to soil-borne inoculum and environmental variables being different between the two sites. In addition, Hare (1997) removed the dead leaf sheaths which may have contained the *M. nivale* infection. At the site where *M. nivale* isolation incidences were variable during the season, initial infection at GS 10-11 was very high, whilst incidence of *F. culmorum* was very low.

Seedling blight

Under favourable conditions, seedling blight can cause pre-emergent and post-emergent death. Seedling blight symptoms caused by *M. nivale* were described by Millar & Colhoun (1969a). Coleoptile lesions occur primarily at the base and tip but some intermediary streaking can occur. Disease severity varies from localised lesions to extensive necrosis and seedling death. Root lesions may also occur. Additional disease symptoms include abnormal radicle or plumule development and leaf lesions. Seedling blight can be caused by seed-borne, soil-borne or debris-borne inoculum (see later sections).

Snow mould

Microdochium nivale causes snow mould when unfrozen ground is covered with snow for several weeks or months. Infected leaves turn yellowish and lie on the ground, before turning brown and necrotic. Attacked roots also become necrotic and the crown may be destroyed. Stunting and deformation of young plants may occur (Cook, 1981).

Foot rot

Foot rot symptoms are brown/black discolourations of the nodes and/or the stem. Foot rot may be an important source of inoculum for ear blight. A correlation ($R^2 = 0.6$) between foot rot intensity of winter wheat in May and average percent seed infection at harvest was recorded during a 12 year survey in Holland between 1974-1986 (Daamen *et al.*, 1991). Foot rot may also directly reduce yield through sterile ears (whiteheads) and lodging. For example, inoculation of glasshouse grown winter wheat (cv. Avalon) at GS 21 by *F. avenaceum*, *F. culmorum*, *F. graminearum* and *M. nivale* mycelial disks at soil level, caused losses in grains ear^{-1}, thousand grain weight (TGW) and yield (Hutcheon & Jordan, 1992).

Foot rot incidence and severity of winter wheat in the UK has been surveyed. Between 1976 and 1988, Polley & Thomas (1991) annually sampled 300-400 winter wheat crops at GS 73-75 in the UK. *Fusarium* diseases were found in at least 74 % of all samples, increasing to over 90 % in several years. However, disease severity was rarely severe and symptoms were only visually assessed, meaning the causal agents of foot rot could not be determined. Investigations which isolated the pathogens from stem-bases (Parry, 1990; Polley & Turner, 1995), have shown that *M. nivale* is an important causal agent of foot rot.

Foliage infection

Microdochium nivale can also infect cereal foliage, although this has not been well investigated. Descriptions of foliar lesions come from two sources. Millar & Colhoun (1969a) reported auburn convex lenshaped spots bordered by a liver-brown band usually on the first but also sometimes the second leaf. Browning of lower leaf sheaths was usually accompanied by perithecium production as flowering started. *Microdochium nivale* var. *nivale* caused triangular-shaped necrotic lesions with downturning of the leaf blade on commercial oats in Ireland (Diamond *et al.*, 1995). The effect of foliage infection is unknown but it is possible it would increase the inoculum potential for ear infection later in the season.

Ear blight

Early ear blight symptoms consist of small brown water-soaked spots on the outer glumes. Under favourable conditions, florets and/or the whole spikelet become infected. Infected tissues lose their chlorophyll and become a bleached straw colour whilst uninfected ears are still green. Pinkish mycelium and conidia may develop in warm humid conditions and purplish coloured perithecia may occur on *M. nivale* infected bracts (Parry *et al.*, 1993). There is little evidence that *M. nivale* grain infection reduces yield. Cassini (1981) in a review of *Fusarium* diseases in cereals in Western Europe proposed without evidence, that *M. nivale* ear blight reduced TGW. In work by Hare *et al.* (1999), the distribution of *M. nivale* infection was similar across all seed sizes for three winter wheat (cv. Riband) seedlots. This limited evidence suggests that if *M. nivale* ear blight infection does reduce yields, it will not be through reduction in TGW. In contrast *F. culmorum* (Doohan *et al.*, 1999) and *F. graminearum* (Wong *et al.*, 1992) can cause large reductions in TGW.

Sources of seedling blight inoculum

Seed-borne *Microdochium nivale*

It is not clear if *M. nivale* infection affects the visual appearance of seeds. *Microdochium nivale* infected seed has been described as shrivelled and discoloured (Millar & Colhoun, 1969a), whilst Hare (1997) and Hare *et al.* (1999) found no difference in the appearance or weight of *M. nivale* infected and pathogen-free seeds. In contrast, *Fusarium* spp. infected grains are lightweight, shrivelled and discoloured red or pink (Chelkowski *et al.*, 1990). Seed-borne *M. nivale* is frequently a problem in the UK. For example, *M. nivale* was found in over 90 % of wheat seed samples tested by NIAB (National Institute of Agricultural Botany) in 1992-3 and 1993-4, over 70 % in 1991-2 and 40 % in 1990-91 (Reeves & Wray, 1994).

There is considerable evidence to demonstrate that *M. nivale* occurs predominantly in the pericarp/endosperm and only occasionally in the embryo of seeds. Bateman (1983) was the first to attempt to discover the location of seed-borne *M. nivale* infection. In this work, seeds of winter wheat (cv. Maris Ranger and cv. Maris Kinsman) after several hours immersion in sterile water were dissected and the seed components plated onto potato dextrose agar (PDA). *Microdochium nivale* was isolated more frequently from the inner pericarp/outer epidermis than the embryo/endosperm. Chelkowski *et al.* (1990) used microscopy and histochemical staining to study wheat grains infected with *M. nivale*. *Microdochium nivale* mycelium was predominantly between the pericarp and aleurone layer and alongside the scutellum but also in the endosperm and embryo. Cristani (1992) employed similar approaches to Bateman (1983). In this work, *M. nivale* was most frequently isolated from the pericarp (34 %) than the endosperm (22 %) and finally the embryo (18 %) of wheat and durum wheat seeds. Sterilisation of the seed components prior to plating changed the isolation occurrence to pericarp 22 %, endosperm 14 % and embryo 4 %. This demonstrated that infection in the pericarp and endosperm was deeper

than embryo infections because a two minute sterilisation would be likely to kill *M. nivale* present only on the surface of the seed components. There have been no attempts to determine if depth of *M. nivale* infection is related to seedling blight severity.

The effect of *M. nivale* infection is shown in final emergence and establishment counts. Surface-borne *M. nivale* (Millar & Colhoun, 1969a) and seeds naturally infected with *M. nivale* (Hewett, 1983) in field trials have reduced final emergence. The extent of *M. nivale* infection is related to the reduction in final emergence and establishment. Percentage seed infection was significantly correlated with field establishment for nine untreated wheat seedlots ($R^2 = 0.971$; Humphreys *et al.*, 1995) and six oat seedlots ($R^2 = 0.831$; Humphreys *et al.*, 1998). Further evidence comes from Hare *et al.* (1999). In pot trials at 22 % soil moisture content, final emergence of three winter wheat (cv. Riband) seedlots decreased from 82 to 48 % of seeds planted, as *M. nivale* infection increased from 19 to 65 %.

Evidence has been presented that reduced seedling emergence and establishment from *M. nivale* infected seedlots is through disease transmission from infected seeds to seedlings, which may die pre-emergence and post-emergence. Rennie *et al.* (1990) demonstrated a good negative correlation ($R^2 = 0.713$) between percentage *M. nivale* infection and percentage germination of 40 *M. nivale* infected seedlots in 1987. However, there was no such correlation for 50 seedlots infected with *M. nivale* from Scotland in 1988. In tray and field trials comprising a range of different environments, significant correlations occurred between *M. nivale* disease incidence on seedlings and the extent of seed-borne infection for nine untreated wheat seedlots (Humphreys *et al.*, 1995) and six oat seedlots (Humphreys *et al.*, 1998).

Further evidence for disease transmission from seeds to seedlings comes from work by Duthie & Hall (1987) in Canada with *F. graminearum*. In a field trial in 1985, significant

correlations occurred between percentage seed infection (0 to 43.5 %) for eight wheat (cv. Frederick) seedlots and plants m^{-2} in October (R^2 = 0.89). However, no such correlations occurred in a subsequent field trial with six wheat (cv. Frederick) seedlots (0 to 14.5 % infection). This implies that environmental conditions during emergence and the extent of seed infection may have significant effects on disease transmission from seed to seedling and subsequent disease severity.

Soil-borne *Microdochium nivale* inoculum

Microdochium nivale can survive and remain pathogenic for long periods in UK soils in the absence of cereals. However, there are many potential sources of inoculum for *M. nivale* and it is difficult to be certain that *M. nivale* is soil-borne rather than debris-borne. *Microdochium nivale* was isolated from mesocotyls and roots of oat seedlings grown from pathogen-free seeds in fields where cereals had not been grown for at least 15 years (Rawlinson & Colhoun, 1969). In a later study, Al-Hashimi & Perry (1986) using barley bait plants found low (3-5 %) infection over the summer months from a field in which barley had been grown the previous year. However, these investigations involved only a few fields in a limited area. Evidence from Al-Hashimi & Perry (1986) also demonstrated that *M. nivale* structures survive in soil for different periods. Conidia of several *M. nivale* isolates retained their pathogenicity to barley seedlings for 25 weeks, mycelium lost its ability to infect after eight weeks, whilst perithecia had no pathogenicity in sterilised soil at 10 °C. However, perithecia did have low pathogenicity three (16 %) and five months (8 %), after burial in the field.

As the aforementioned work has demonstrated, soil-borne *M. nivale* can cause seedling blight but rarely gives rise to serious attacks on UK cereals. Colhoun (1972) first proposed this without any evidence. In fifteen early (September and October) drilled multi-site field trials between 1992 and 1994 in England and Wales, untreated, pathogen-free, high vigour

seeds did not show reduced final emergence compared to treated seeds (Paveley & Davies, 1994). However, it is likely that soil-borne disease pressure would only cause significant seedling death under poor environmental conditions associated with later drilling dates.

There is evidence that soil-borne *M. nivale* only causes severe seedling blight under conditions causing slow seedling growth. In field soil maintained at 75 % maximum water holding capacity (MWHC) under a 10 / 2 °C diurnal temperature regime, *M. nivale* was frequently isolated from oat seedlings from untreated and organomercury treated pathogen-free seeds (Rawlinson & Colhoun, 1969). This demonstrates that soil-borne *M. nivale* can infect seedlings from organomercury treated seeds under conditions typical of UK winters. However, no lesions were present on the seedlings and no additional measure of disease, such as loss of vigour, was assessed. Therefore, it is not clear if *M. nivale* was adversely affecting seedling growth.

Debris-borne *Microdochium nivale*

There is evidence that *M. nivale* can survive in plant debris. *Microdochium nivale* saprophytically colonised wheat leaves, roots and stem pieces in artificially-inoculated moist soil at 10, 15 and 20 °C (Booth & Taylor, 1976b). Al-Hashimi & Perry (1986) demonstrated that the location of debris in the soil is important. *Microdochium nivale* survived longer in buried straw than in straw on the soil surface over a 15 week period in sterilised soil at 15 °C.

Debris-borne *M. nivale* can cause seedling blight. Booth & Taylor (1976a) found *M. nivale* in straw could reduce field establishment of winter wheat. However, when stubble was removed prior to ploughing, ploughed-in, or left standing prior to drilling, there was no effect on final emergence or survival of spring wheat (cv. Jufy I) and winter wheat (cv. Viking). This indicates that debris-borne *M. nivale* does not always cause seedling

blight, possibly due to low amounts of inoculum or unfavourable environmental conditions for infection and disease expression. Further evidence for debris-borne *M. nivale* causing seedling blight comes from Booth & Taylor (1976b). *Microdochium nivale* in saprophytically colonised leaf, root and straw portions caused seedling blight of wheat in 46 % MWHC soil at 15 °C.

In summary, seed-borne *M. nivale* appears to be the most common source of seedling blight in the UK. Seedling blight may occur from soil-borne and debris-borne *M. nivale*, but is rarely common or severe, except when large amounts of inoculum are present under favourable environmental conditions.

Environmental conditions affecting *Microdochium nivale* seedling blight

There is considerable evidence that the most severe *M. nivale* seedling blight occurs under conditions which slow seedling emergence. Only Hare *et al.* (1995) have investigated the combined effects of soil moisture and temperature on seedling blight. In pot trials, lowest final emergence from a winter wheat (cv. Riband) seedlot with 72 % *M. nivale* infection occurred in cold dry soil between 12 and 6 °C and -5, -36 and -108 KPa water potential. A significant effect of soil moisture and temperature on final emergence was reported but there was no interaction between the two variables. Under all experimental conditions, slower seedling emergence reduced final emergence. A similar trend was observed for winter wheat (cv. Mercia) surface-inoculated with *F. culmorum* spores (Hare & Parry, 1996).

There is evidence that cold dry soils also increase seedling blight severity from surface-borne *M. nivale* inoculum. The most severe disease indices and greatest reductions in dry weight, occurred at low temperatures between 6.1 and 16.4 °C at 8.8, 14.0, 19.3 and 24.5 % w/w soil moisture for winter wheat (cv. Atle) surface-inoculated with 5000 spores

seed^{-1} in glasshouse experiments (Millar & Colhoun, 1969b). Soil temperature appeared to be a more important determinant of seedling blight than soil moisture because at all moisture regimes, disease index and dry weight reductions were greater at reduced temperatures. However, the maintenance and control of soil temperature and moisture regimes was not fully described. Cassell & Hering (1982) described increased disease symptoms on leaf sheaths of seedlings from wheat (cv. Cappelle-Desprez) seeds surface-inoculated with 2500 *M. nivale* spores per seed grown in dry (-50 KPa) rather than wet soil (-5 KPa). Disease incidence and severity on sheaths was slightly greater at 12 than 15 °C. However, the soil was not sterilised in this investigation, allowing soil-borne *F. culmorum* to interfere with the disease assessments.

These investigations have indicated that temperature and soil moisture are important determinants of *M. nivale* seedling blight. Spore number per seed is also a significant factor affecting disease severity. High *F. culmorum* spore numbers per seed caused more severe seedling blight on winter wheat (cv. Atle) over a wider range of soil temperatures and soil water regimes than reduced spore loads (Colhoun *et al.*, 1968). It is possible that surface-borne inoculum is affected more by temperature and moisture, therefore, experiments with naturally infected seeds are more representative. There is currently a lack of research into seedling blight using naturally infected seed under fluctuating conditions.

In summary, it is unclear why increased seedling blight incidence and severity occurs under adverse seedling growing conditions. Only Bennett (1933) described death of wheat, barley, oat and rye seedlings from soil-borne and seed-borne *M. nivale* inoculum under mainly adverse conditions in pot trials. Despite Bennett not describing the 'good' growing conditions, subsequent investigations using pathogen-free seeds have demonstrated that seedlings do grow better under certain conditions (see page 21). It is

therefore likely that increased seedling blight arises under conditions that increase *M. nivale* pathogenicity, whilst also retarding seedling growth.

Effect of temperature on *Microdochium nivale* seedling blight

Microdochium nivale grows best at low temperatures. Evidence for this comes from Booth & Taylor (1976b). Saprophytic growth of *M. nivale* through non-sterile soil from wheat seed and straw inoculum was faster at 10 than 20 °C. However, it was not apparent whether this was due to increased *M. nivale* growth or reduced soil micro-flora competition and inhibition at 10 °C. There is evidence from work by Perry (1986) that soil-borne *M. nivale* is also more pathogenic to seedlings at low temperatures. In sterilised soil infested with *M. nivale* conidia, more barley coleoptiles were infected with increased severity at 10 than 20 °C. These investigations imply that increased pathogenicity at low temperatures is due to increased *M. nivale* growth. Similar observations have not been reported for field trials using soil-borne *Fusarium* spp. In work by Paveley & Davies (1994), mean soil temperature (obtained from the nearest available meteorological station) from drilling to ten days after drilling, was not correlated to final emergence across nine sites of wheat and six sites of barley between 1992 and 1994. However, seedling blight disease severity was not measured.

Seed-borne *M. nivale* is also more pathogenic to seedlings under low temperatures. Germination of ten wheat cultivars infected with *M. nivale* was greater at 12 h:25 °C; 12 h:15 °C, than 6 h:15 °C; 18 h:10 °C (Cristani, 1992). Numbers of seeds or seedlings infected with *M. nivale* were also reduced for the warmer regime. Additional evidence came from Hare *et al.* (1995) where final emergence was shown to decrease as constant temperatures were lowered between 12 and 6 °C for four winter wheat (cv. Riband) seedlots with 4, 8, 19 and 44 % *M. nivale* infection.

The reason for increased seedling blight severity at low temperatures is not known. Cristani (1992) proposed that seedling blight is unlikely to occur at temperatures which favour quick seedling growth. This is supported by work on *F. graminearum*. Germination of two untreated spring wheat cultivars surface-inoculated with *F. graminearum* conidia, was lowest when germinated at temperatures optimum for *F. graminearum* growth in pot trials. Conversely, germination was best at temperatures outside the growth range of *F. graminearum* (Dickinson, 1923). Similar findings were reported for seeds of two spring wheat cultivars infected with *F. graminearum* in glasshouse pot trials (Gilbert *et al.*, 1997).

Methods for measuring soil water content

There are several techniques for measuring soil water content. Tensiometers measure soil water content by allowing the water in the soil to come into equilibrium with a reference pressure indicator through a permeable ceramic cup in contact with the soil. However, this method is not completely accurate in dry soils. A psychrometer uses a thermocouple to measures the difference between a reference junction and the soil sample. This method measure the availability of soil water to plants but is affected by temperatures. A capacitance probe generates an AC field that can detect changes in soil dielectric properties which are linked to variations in soil water content. This method is very accurate across all soil water contents but is very dependent on good sensor tube-soil contact. Neutron probes emit neutrons which lose energy when they encounter hydrogen in soil water. The de-energised neutrons are detected by a gas that can be neutron activated. This neutron activated gas is detected using an electronic device. The number of thermalised neutrons is proportional to the soil water content. This method is very accurate at deep depths but has reduced accuracy close to the soil surface. A further approach to measuring soil water content is weight loss after oven drying. This method is very accurate and repeatable across all soil water contents. In this method a soil sample is weighed, dried at 102 ^{o}C to a

constant weight and re-weighed. It is assumed the difference between the wet and dry weight is the weight of water in the sample which is expressed as a percentage of the dry soil weight.

Effect of soil water on *Microdochium nivale* seedling blight

There is limited evidence from pot trials by Millar & Colhoun (1969b) that seedling blight from seed surface-borne *M. nivale* is more severe in dry soils. In this work, increasing soil moisture from 8.8 to 24.5 % w/w reduced disease index on winter wheat coleoptiles/culms and increased seedling dry weight across all temperature regimes (6.1 to 16.4 ^{o}C). Hare *et al.* (1995) demonstrated greater final emergence at -5 than -108 KPa water potential for winter wheat (cv. Riband) with 72 % *M. nivale* seed infection.

Far more investigations have been conducted into the effect of soil moisture on *F. culmorum* seedling blight. *Fusarium culmorum* seedling blight from surface-inoculated seeds is also more severe in dry soils. When soil moisture contents were maintained by addition of water to a pre-determined amount, *F. culmorum* caused most severe pre-emergent and post-emergent death in warm dry soils (Colhoun & Park, 1964). Cassell & Hering (1982) maintained constant water potentials using tension tables. Increased seedling blight severity occurred on wheat seedling sheaths at -47 to -50 KPa rather than -7 to -7.5 KPa at 15 and 20 ^{o}C. However, limitations in the experimental design meant water content variations of up to 5 % occurred. In pot trials at 20 ^{o}C, pre-emergent seedling death of winter wheat (cv. Mercia) increased from 25 to 55 % in direct proportion to mean soil matric potential (Ψ_m) between -0.14 and -0.17 MPa (Hare & Parry, 1996). The percentage of seedlings showing post-emergent symptoms was inversely related to mean Ψ_m.

Additional evidence for the importance of soil water in seedling blight comes from investigations by Liddell & Burgess (1988). In this work, constant soil moisture potentials were maintained in wax partitioned soil columns using pressure membrane apparatus or isopiestic equilibration. Increased isolation from wheat coleoptiles grown in soil containing *F. graminearum* inoculum at 20-22 °C occurred in dry soil. Seedling infection occurred between saturation and -2.15 MPa. Greatest infection incidence (92 %) occurred at -0.3 MPa.

From all the aforementioned investigations it is not possible to determine whether soil moisture is affecting the pathogen or seedling growth. There is evidence to indicate that increased seedling blight incidence and severity in dry soils is due to slower seedling growth. Increased winter wheat (cv. Viking) germination and subsequent growth appeared to reduce the opportunity for *F. culmorum* infection from surface-borne inoculum (Malalasekera & Colhoun, 1968). Soaking seeds for three hours had the greatest effect on increasing rate of seedling emergence, mean root length and weight, and reducing seedling blight severity at GS 13 in pot trials and reducing seedling blight severity in field trials. Seedlings from seeds soaked for three days had increased disease severity probably due to depressed seedling vigour, increasing the opportunity for infection. In addition, Hare & Parry (1996) demonstrated a good relationship between mean soil water potential and seedling emergence rate but did not consider *F. culmorum* growth rate.

Effect of *Microdochium nivale* seedling blight on yield

Compensatory tillering can lessen the effect of reduced seedling establishment caused by *M. nivale* on yield. Evidence for the effect of *M. nivale* seed-borne infection on reduced establishment and yield comes from the work of Humphreys *et al.* (1995). Establishment of nine untreated winter wheat cultivars infected with *M. nivale* and *Fusarium* spp. in a field trial was significantly correlated with yield ($R^2 = 0.728$). Similar correlations also

occurred between establishment and yield (R^2 = 0.768) for six oat cultivars infected with *M. nivale* (Humphreys *et al.*, 1998).

Non-lethal seedling blight may act as a source of inoculum for foot rot. Evidence for this comes from the work of Perry (1986). In two years of field trials with spring barley (cv. Golden Promise) seeds surface-inoculated with *M. nivale* spores, stem-base infections were more frequent on seedlings from untreated seeds than seedlings from triadimenol + fuberidazole treated seeds at three sampling dates during the season.

Effect of temperature and soil water on wheat seedling growth from pathogen-free seeds

The effect of temperature and soil water on early seedling growth from pathogen-free seeds has been extensively studied. The first stage of emergence is imbibition. Wheat seeds must reach a critical moisture content (approximately 30 % increase in seed weight) before germination can begin. Rate of water uptake but not the final amount, is dependent upon temperature and moisture availability. Rate of water uptake for wheat (cv. Thell) seeds dramatically increased with increased soil water content between 8.1 and 13.6 % at 22 °C (Dasberg, 1971). Chaudhary *et al.* (1971) found that final water absorption of wheat seeds in soil was not significantly different between 5 and 35 °C. Again rate of water absorption by seeds increased as the temperature increased.

After imbibition, germination occurs in favourable conditions. Wheat germinates better at higher temperatures and in moist conditions. Owen (1952) first investigated the effect of water potential on germination. At 20 °C, more uniform and increased germination of Squareheads Master wheat seeds occurred at high water potentials. Irregular and reduced germination occurred at lower water potentials. There is also evidence that conditions that reduce germination also slow it down. Rate of germination for six American winter wheat

cultivars increased with rises in constant temperatures between 5 and 25 °C (Kamaha & Maguire, 1992). Germination declined rapidly below 13 °C and was zero for most cultivars at 5 °C. Reasons for this are not apparent since winter wheat will typically germinate and grow at and below 5 °C in the UK.

Reduced temperatures and reduced soil water potentials slow the rate of germination. During work by Lafond & Baker (1986) with nine spring wheat cultivars, median germination time was 90 h at 0 MPa and 156 h at -0.8 MPa at 10 °C. At 20 °C, median germination time increased from 36 h at 0 MPa, to 64 h at -0.8 MPa. Maximum germination of four wheat cultivars occurred at 18 to 22 °C between -0.3 and -1.8 MPa. Rate of germination was slower and final germination reduced at temperatures below 10 °C and osmotic potentials below -0.3 MPa (Hampson & Simpson, 1990). In work by Wuest *et al.* (1999) temperature (3 to 28 °C) and water potential (-0.15 to -1.1 MPa) significantly reduced time to germination of wheat (cv. Madsen) only in the coldest (3 °C) and driest (-1.1 MPa) soils.

Emergence is complete after the coleoptile elongates and penetrates the soil surface and is dependent on temperature and soil moisture. In pot trials by Lindstrom *et al.* (1976), rate of emergence for winter wheat (cv. Nugaines and cv. McCall) progressively decreased as constant water potential was reduced between -40 and -2 MPa. Lowering the constant temperature from 25 to 5 °C also slowed the rate of emergence. DeJong & Best (1979) reported that 50 % emergence of wheat (cv. Canthatch) was slowed when temperature was reduced from 26.7 to 5 °C at constant water potentials (-33, -0.6 and -1 MPa). However, accumulated day degrees (°Cd) required for final emergence, were not significantly different between the temperature and moisture regimes. In pot trials, a similar relationship occurred between 10 and 18 % constant soil moisture contents and constant soil temperatures of 8 to 20 °C. Interestingly, in all instances, there was no interaction

between soil moisture and temperature on seedling emergence. Khah *et al.* (1986) investigated the effect of soil water content and temperature on emergence under field conditions. Probit percentage final emergence of three spring wheat seedlots (cv. Timmo) had a positive linear relationship with mean soil temperature between 7 and 11 °C and a negative relationship with soil moisture contents between 12.1 and 15.5 %.

Ashraf & Abu-Shakra (1978) demonstrated that respiration rates of four wheat cultivars were inversely related to moisture stress (between 0 and -1.8 MPa) at 3 °C:16 h; 10 °C:8 h or 6 °C:16 h; 15 °C: 8 h. Therefore, low temperature and moisture stress may slow down emergence by retarding seedling growth. For example, coleoptile extension of seven winter wheat (cv. Slejpner) seedlots was reduced at low osmotic water potentials between 0 and -1.15 MPa at 20 °C (Naylor & Gurma, 1990). At -5.9 KPa Ψ_m, rate of winter wheat (cv. Rosella) and spring wheat (cv. Hartog) coleoptile elongation was directly related to constant temperatures between 5 and 25 °C (Addae & Pearson, 1992).

Wheat seedlings emerge faster from warm (>10 °C) moist (>-0.3 MPa) soils. Low temperatures (<10 °C) and dry soil (<-0.3 MPa) conditions may interact and reduce rate of emergence and final emergence. Conditions that retard seedling growth increase the severity of seedling blight caused by *M. nivale*. Although the precise reasons for this observation are unknown, it could be postulated that cold dry soils which slow seedling growth increase the opportunity for *M. nivale* to infect and cause damage. It is possible that the interactions between *M. nivale*, the seedling and the environment are very important.

Chemical control of *Microdochium nivale* seedling blight

Seedling blight in the UK is controlled by fungicide seed treatments. Seed treatments increase final emergence above untreated seeds and reduce seedling blight incidence and

severity from seed-borne (Millar & Colhoun, 1969b; Jones, 1993) and soil-borne (Rawlinson & Colhoun, 1969) *M. nivale* inoculum.

There is limited evidence to suggest that control of seed-borne *M. nivale* occurs through direct contact between fungicide and pathogen. As such, it could be argued that infection in the seed coat would be controlled before that located deeper in the seed. Components of winter wheat seed (cv. Maris Ranger and cv. Maris Kinsman) after soaking in water for seven hours were dissected and plated onto PDA. The incidence of *M. nivale* recovery from the outer epidermis and inner pericarp/seed coat of phenyl mercuric acetate treated seeds was significantly lower than from untreated seeds (Bateman, 1983). There was no effect on infection located in the endosperm and embryo, possibly due to the limited time interval preventing the fungicide from penetrating deeper into the seed. Hutcheon & Jordan (1992) presented evidence that seed treatments reduce disease from soil-borne *M. nivale* by reducing fungal penetration and subsequent colonisation. In out-door pot trials, eight seed treatments reduced stem and leaf lesions and colonisation of wheat (cv. Avalon) seedlings at GS 37.

Effect of environmental conditions on seed treatment efficacy

There is evidence to suggest that variability in seed treatment performance is due to environmental factors. Whilst the effect of environment on *M. nivale* has been discussed previously, it is possible that environmental conditions may also influence seed treatment performance. Paveley & Davies (1994) highlighted the variable performance of seed treatments on pathogen-free seedlots. At different sites throughout the UK, final emergence of winter wheat, treated with six seed treatments was variable across nine sites. A similar trend occurred across six sites for winter barley treated with six seed treatments. Reasons for this are not readily apparent.

The effects of environmental conditions on seed treatment efficacy towards *M. nivale* infected seedlots have also been demonstrated. Final emergence of a wheat seedlot with over 70 % infection, treated with triadimenol + fuberidazole, fenpiclonil, carboxin + thiabendazole, guazatine and mercury treatments was different at two sites in the UK between 1991-1992 (Noon & Jackson, 1992). In further work by Morris *et al.* (1994), triadimenol + fuberidazole, phenylmercury acetate ammonium complex, carboxin + thiabendazole, guazatine and flutriafol + thiabendazole performance was variable, measured by final emergence from infected winter wheat seedlots and reductions in stem infections, over six seasons of field trials between 1988-1993. However, in further trials at four UK sites, triadimenol + fuberidazole and guazatine performed similarly, increasing final emergence and reducing stem infections at GS 12-13 on two winter wheat cultivars with unspecified infection (Morris *et al.*, 1994). Work by Cox & Mussard (1994) also showed similar seed treatment efficacy across sites. Triadimenol + fuberidazole, carboxin + thiabendazole and guazatine treatments increased final winter wheat emergence from seedlots with infection between 48 and 60 % similarly across three English sites in 1992. No measure of environmental conditions was made in these investigations, therefore it is difficult to determine what environmental variables are influencing performance of seed treatments.

There is evidence from pot trials that disease pressure does not affect seed treatment performance (Bateman, 1977). Organomercury performance was similar against 12.5 and 25 g *F. culmorum* mycelium per kg soil, despite increased disease severity on coleoptiles from untreated wheat seeds at 26 % soil moisture content and 13 / 8 ^{o}C diurnal temperature regime. Hare *et al.* (1995) demonstrated that the extent of *M. nivale* seed infection had no effect on final emergence from winter wheat treated seeds albeit with a limited number of seedlots.

Initial evidence from Bateman (1976) indicated that low temperatures had no effect on organomercury efficacy. Maintaining newly emerged wheat and barley seedlings from naturally infected organomercury treated seeds at 0-1 °C did not increase the *M. nivale* isolation frequency from coleoptiles compared to plants grown at 13 / 8 °C. However, later work by Hare *et al.* (1995) demonstrated that temperature can significantly affect the efficacy of some seed treatments, measured by final emergence from a 72 % *M. nivale* infected winter wheat (cv. Riband) seedlot. In controlled environments, thiabendazole performance was very poor at 6 and 8 °C, flutriafol performance declined noticeably at reduced temperatures, whilst prochloraz and guazatine performed similarly across all temperatures.

Soil moisture appears to have little effect on seed treatment efficacy. Organomercury treatment reduced the severity of seedling blight on winter wheat (cv. Viking) grown from seeds with 30 % *M. nivale* infection and winter wheat (cv. Atle) inoculated with 5000 spores seed^{-1} at constant soil water contents between 8.9 and 23.9 % at 10 °C mean temperature (Millar & Colhoun, 1969b). Reductions in disease indices on winter wheat (cv. Joss Cambier) seedlings from soil-borne *F. culmorum*, by organomercury treatment compared to untreated seeds was not related to soil water contents of 26, 28 and 31 % (Bateman, 1977). In summary, variable seed treatment performance, measured by final emergence and seedling blight incidence and severity, across sites is probably due to environment effects, specifically temperature and soil water content, on the seedling, the seed treatment and *M. nivale*.

Effect of seed treatments on subsequent winter wheat growth and yield

There is conflicting evidence regarding the effect of seed treatments on yield of winter wheat in the UK (Richardson, 1986; Jackson *et al.*, 1994; Paveley & Davies, 1994). Whether seed treatments increase yield is probably determined by the extent of *M. nivale*

infection, drilling rate and environmental conditions, which will affect possible plant compensation. Seed treatments increase yields when seedling loss from untreated *M. nivale* infected seedlots is severe and there are too few plants remaining to compensate for killed seedlings. Noon & Jackson (1992) first reported this in a field study in 1991-1992. Increased establishment from fungicide treated seeds over untreated seeds at one site gave a greater yield response over another site, where increased establishment over untreated seeds was less. Hare (1997) recorded a similar occurrence at two UK sites in 1993-1994. Fungicide seed treatments only significantly increased yield at the site where establishment from untreated *M. nivale* infected seeds was much reduced. Therefore, it appears that the key point for seed treatments increasing yield is a significant increase in establishment.

Seed treatments can reduce foot rot and ear infections, possibly through lowered inoculum densities. Visible *Fusarium* ear blight symptoms from natural infection were significantly reduced in wheat grown from seeds treated with carboxin + thiram in Canadian field trials at three sites in 1984 (Teich & Hamilton, 1985). In controlled environment investigations by Hutcheon & Jordan (1992), eight seed treatments reduced disease severity on the top three wheat internodes from soil-borne *M. nivale* inoculum. However, only three seed treatments significantly reduced the diseased ear area and none of the seed treatments significantly increased yield.

Effects on crop health will depend on the seed treatment and the source of inoculum. In American field trials over two years, carboxin, maneb and triadimenol did not significantly reduce the incidence of crown infection of winter wheat in spring or summer from soil-borne *Fusarium* spp. (Celetti & Hall, 1987). This was probably because seed treatment concentrations were much reduced at the time of disease challenge. Therefore, the environment and disease pressure during the season will have important effects on potential seed treatment improvements on crop health and yield.

Aims of the project

The aims of this investigation were to:

- *Predict field conditions likely to cause severe* M. nivale *seedling blight*

The hypothesis that fluctuating soil water contents and soil temperatures affect seedling blight severity, caused by *M. nivale* was challenged. This was achieved by monitoring soil temperatures in nine field trials in one year. In two subsequent years, temperature and soil water content were monitored in field trials at Harper Adams that involved a range of drilling dates providing different seedbed conditions.

- *Elucidate the mechanisms of seedling infection from seed-borne* M. nivale

The hypothesis that seed-borne *M. nivale* infects the seedling during early seedling growth was tested through controlled environment studies comprising a range of temperatures and soil water regimes.

- *Study the interaction between temperature and soil water content on development of seedling blight caused by* M. nivale

In vitro and quantitative PCR techniques were used to characterise in more detail, the host-pathogen interaction at various temperatures and soil water regimes. The hypothesis that low temperatures (5 °C) and reduced soil water contents favour *M. nivale* growth, whilst higher temperatures (>10 °C) and high soil water contents favour seedling growth was tested.

- *Determine the effect of sub-zero temperatures on seedling emergence and seedling blight severity from* M. nivale *infected seedlots*

Controlled environment studies were conducted to test the hypothesis that the timing and duration of freezing had no effect on seedling blight. A further hypothesis that the timing and duration of freezing has no effect on the efficacy of carboxin + thiram seed treatment was also challenged.

- *Establish the effect of seed-borne* M. nivale *infection on foot rot disease and stem colonisation, subsequent plant growth and yield*

The hypothesis that the extent of seedling blight severity affects subsequent plant growth and stem colonisation was tested in glasshouse studies by destructive sampling at GS 40-49 and harvest. A second hypothesis that seed-borne *M. nivale* does not cause foot rot was also tested.

- *Determine the effects of a range of seed treatments on seedling emergence, plant growth, stem-base disease and yield under field conditions*

This was achieved through two years of field trials at Harper Adams with multiple drilling dates in each year to challenge the hypothesis that seed treatment effects on final emergence, establishment and seedling blight disease severity at GS 10-12 and GS 15-25 were not affected by fluctuating soil temperatures and soil water contents. A further hypothesis that seed treatments have no beneficial effect on plant productivity after GS 15-25 under a range of environmental conditions was challenged by taking measurements of shoots m^{-2}, ears m^{-2}, yield and TGW and foot rot disease incidence at GS 40-49 and GS 75.

CHAPTER 3

General Materials & Methods

GENERAL MATERIALS & METHODS

Aseptic techniques

Glassware, pathogen growth media, distilled water and filter paper were autoclaved (121 °C: 108 KPa) for 20 min. Aseptic operations were performed in a laminar flow cabinet with surfaces sterilised with alcohol prior to use.

Surface-sterilisation

Plant material was placed in a solution of sodium hypochlorite (1 % available chlorine) (BDH Chemicals Ltd. Poole, UK) containing 0.05 % v/v Tween 20 (Sigma-Aldrich Company Ltd, Dorset, UK) for 3 min, washed three times in sterile distilled water, placed on sterile filter paper and dried in a flow of sterile air.

Soil water

Determination of field soil water content

Eight soil cores (7.5 cm diameter x 5 cm depth) were taken from guard plots around each trial, weighed and oven dried at 102 °C to a constant weight (Anon., 1977). Each sample was then reweighed and passed through a 2 mm sieve. The weight of any stones larger than 2 mm was subtracted from the soil weight. Percentage soil water content by mass was calculated (Equation 1).

Determination of soil water content for controlled environment investigations

Five 30 g samples of compost for use in controlled environment trials were oven dried at 102 °C to constant weight (Anon., 1977). Each sample was then reweighed and the % w/w soil water content calculated (Equation 1).

$$\text{soil water content (\% w/w)} = \frac{\text{weight of wet soil} - \text{weight of dry soil}}{\text{weight of dry soil}} \times 100 \qquad \textbf{Equation 1}$$

Water potential

Water potentials of polyethylene glycol (PEG) 4000 (BDH Chemicals Ltd) solutions at 15, 10 and 5 °C were determined using a HR-33T Dewpoint Microvoltmeter (Wescor Inc., Logan, Utah, USA) according to the method of Michel & Kaufmann (1971).

Soil temperature

Soil temperature at seed depth was recorded every 15 minutes using Gemini data loggers (Chichester, West Sussex, UK). These data were used to determine hourly median temperatures and subsequently calculate median daily temperatures.

Host cultivars

Source, year of harvest, cultivar, percentage germination and percentage *M. nivale* seed-borne infection of winter wheat (*Triticum aestivum* L.) seedlots are given in Table 2. The moisture content of each seedlot was between 10-14 %. Each seedlot was screened to ensure all seeds were between 2 and 4 mm and stored in a sealed plastic bag at 5 °C. Seed germination was assessed using the tetrazolium biochemical test according to the international rules for seed testing (Anon., 1985). Seed-borne *M. nivale* infection was determined from two replicates of 100 surface-sterilised seeds (refer to seed-borne disease incidence). The number of colonies from seeds was expressed as a percentage of the total seed number.

Culture of host

Host growth media

Seeds were planted into a sandy loam soil-based John Innes No. 2 compost (Taylors Organex, Crewe, Cheshire, UK). Before planting, the compost was passed through a 5 mm sieve and autoclaved (121 °C: 108 KPa) for 60 min on three consecutive days.

Table 2. Cultivar, percentage germination and percentage *Microdochium nivale* infection of winter wheat seedlots used.

seedlot	cultivar	harvest year	source*	% germination	% infection
1	Cadenza	2001	HAUC	87	0
2	Cadenza	2001	HAUC	81	29
3	Equinox	2001	HAUC	91	0
4	Equinox	2000	HAUC	78	8
5	Equinox	2000	HAUC	95	45
6	Equinox	2000	HAUC	88	88
7	Hereward	1997	Scotland	91	55
8	Riband	1998	Scotland	91	30
9	Riband	1998	Scotland	74	56
10	Riband	1999	Scotland	95	73
11	Unknown	1998	Scotland	94	36

* HAUC = Harper Adams University College
Seedlots 7, 8, 9, 10 and 11 obtained from Crompton Europe Ltd

Planting of seed

Surface-sterilised seeds were planted at 20 mm depth. See Table 3 for details of the containers, compost quantities used and the number of seeds planted. Seed trays were surface-sterilised by immersion in sodium hypochlorite solution (1 % available chlorine) for 10 min, rinsed with sterile water three times and air-dried prior to use. Glass jars and lids were autoclaved (121 °C: 108 KPa) for 20 min prior to use.

Table 3. Details of containers used, compost amounts and seed rates.

container	dimensions (mm)	compost amount	seed number per container
Plastic ¼ seed tray (Ward, Wednesbury, West Midlands, UK)	215 x 155 x 50	1000 g	50 or 100
250 ml clear glass jar, wide screw neck (Fisons, Loughborough, Leicestershire, UK)	60 x 60 x 120	80 g	10

Maintenance of soil water

Soil in trays was watered to a pre-determined weight using sterile distilled water applied through a fine nozzle. Soil in sealed glass jars was not watered.

Assessment of rate of seedling emergence

The number of emerged seedlings was counted daily. Final emergence was defined as complete when no further seedlings emerged for five consecutive days. The mean seedling emergence time (in days) was calculated using Equation 2 (Khah *et al.*, 1986). Mean rate of emergence (day^{-1}) is the reciprocal of the mean seedling emergence time.

$$\text{mean seedling emergence time (day)} = \frac{\sum (D \times n)}{\sum n} \qquad \textbf{Equation 2}$$

where n is the number of seedlings which emerged on day D (number of days after planting).

Microdochium nivale disease assessment

Seed-borne disease incidence

Five surface-sterilised seeds were placed crease down into a 90 mm Petri dish containing PDA amended with 130 μg ml^{-1} agar streptomycin sulfate (Sigma-Aldrich Company Ltd) and 25 μg ml^{-1} agar carbendazim 50 % w/w (BASF, Bury St. Edmunds, UK) and incubated

at 15 °C. After 10-14 days, *M. nivale* colonies were identified by their colour and spore morphology (Booth, 1971).

Seedling blight disease assessment

Disease severity was assessed by:

1. pre-emergent death (non-emergence) at GS 10-12.
2. post-emergent death at GS 15-25.
3. a necrosis assessment on emerged seedlings at GS 10-12 and GS 15-25. Seedlings were assigned one of five disease values (0, 1, 2, 3 or 4) according to symptom severity (Table 4) and a disease index calculated (Equation 3).

Table 4. Descriptive key used for the assessment of *Microdochium nivale* seedling blight disease severity (after Hare, 1997).

score	GS 10-12	GS 15-25
0	no symptoms	no symptoms
1	1-2 lesions on coleoptile	1-2 lesions on stem-base
2	more than 2 lesions on coleoptile	more than 2 lesions on stem-base
3	total necrosis of coleoptile	total necrosis of stem-base
4	total necrosis of coleoptile and deformed seedling growth	total necrosis of stem-base and deformed seedling growth

$$\text{disease index} = \frac{(a \times 0) + (b \times 1) + (c \times 2) + (d \times 3) + (e \times 4)}{(a + b + c + d + e) \times 4} \times 100 \qquad \textbf{Equation 3}$$

where a, b, c, d and e are the number of seedlings in classes 0 to 4 respectively.

Foot rot assessment

Foot rot incidence was assessed from 25 plants per field plot. Dead leaf sheaths were removed from each shoot and the stem assessed for browning between the roots and the first node. Plants were classified as diseased or not diseased. Disease incidence was calculated using Equation 4.

$$\text{foot rot incidence (\%)} = \frac{(a \times 0) + (b \times 1)}{25} \times 100 \qquad \textbf{Equation 4}$$

where a = number of disease-free plants and b = number of diseased plants.

Dry weight assessment

Plant matter was dried at 102 ± 2 °C for 24 h in a forced draught system oven, weighed and expressed as dry weight per plant.

Competitive PCR

Samples were crushed and DNA extracted in cetyltrimethylammonium bromide (CTAB) buffer (Appendix A) at 65 °C for 16 h. Potassium acetate (5M) was added, tubes mixed, frozen at -20 °C, thawed, mixed and centrifuged at 3000 *g* (Beckman Model TJ-6 Centrifuge; Beckman Coulter (UK) Ltd, Bucks, UK) for 15 min. Some of the supernatant (1.3 ml) was removed and added to 0.6 ml chloroform, vortexed and spun at 12000 *g* (Heraeus Sepatech Biofuge 13; Heraeus Instruments Ltd, Essex, UK) for 15 min. One ml of the aqueous phase was pipetted into 0.8 ml isopropanol, mixed again and incubated at 18 °C for 30 min. Samples were centrifuged at 6000 *g* for 15 min, the supernatant discarded and pellet washed twice with 44 % isopropanol. Pellets were air-dried in a fumehood (Big Neat Ltd, Hants, UK) and resuspended in 200 µl TE buffer (Appendix A) at 65 °C for 1 h before storage at 4 °C. Total DNA was quantified by spectrophotometry (Beckman Instruments, Fullerton, California, USA) based on absorbance at 260 and 280 nm and samples diluted to a DNA concentration of 40 or 4 $ng\ \mu l^{-1}$ if the band intensities were above the range of the standard curve. Internal standards and *M. nivale* specific primers (developed by Dr S.G. Edwards) were used in competitive PCR reactions to quantify amounts of *M. nivale* DNA as detailed previously (Edwards *et al.*, 2001).

Pathogen DNA in 50 μl PCR mixtures (Appendix A) was amplified in a PTC-100 Thermal Cycler (MJ Research, Massachusetts, USA). The PCR program had 35 temperature cycles of 94 °C for 15 s, 66 °C for 15 s and 72 °C for 45 s. The first cycle had an extra 75 s at 95 °C and the final cycle an extra 4 min 15 s at 72 °C.

PCR products were observed after electrophoresis through agarose gels (2 % w/v agarose containing 0.5 μg ethidium bromide ml^{-1}) in TAE buffer (Appendix A). Gels were viewed under UV light using a Gel Doc 1000 fluorescent gel documentation system (Bio-Rad Laboratories Ltd, Hemel Hempstead, UK). PCR product ratios were determined for the standard and sample by dividing the band intensity of the sample by that of the internal standard. DNA quantities were expressed as *p*g fungal DNA *n*g^{-1} total DNA.

Seed treatment application

Seed treatments were applied at the manufactures' full recommended dose rates (Table 5) using a Mini-Rotostat (Marline Ltd, Norfolk, UK).

Table 5. Trade name, manufacturer, active ingredient(s) and application rate of fungicide seed treatments used to control *Fusarium* seedling blight.

trade name	manufacturer	active ingredients (g l^{-1})	use rate (l tonne seed^{-1})
Anchor	Crompton Europe Ltd	200 carboxin + 200 thiram	3.0
Beret Gold	Novartis Crop Protection	25 fludioxonil	2.0
Jockey	Aventis CropScience	167 fluquinconazole + 31 prochloraz	4.5
Panoctine	Aventis CropScience	300 guazatine	2.0
Sibutol	Bayer CropScience UK	375 bitertanol + 23 fuberidazole	1.5

All seed treatments were flowable concentrates except Panoctine (solution).

CHAPTER 4

Effect of seed-borne *Microdochium nivale* infection on the early phases of seedling growth under a range of temperatures and soil water regimes

INTRODUCTION

Seed-borne *M. nivale* infection reduces final emergence of winter wheat when untreated seedlots are grown under conditions conducive to disease development (Hare *et al.*, 1995). Little work has considered the effects of *M. nivale* infection on early seedling growth and development (imbibition, germination, emergence and first leaf events). Millar & Colhoun (1969b) during a field study showed that seedlings grown from wheat seeds artificially-inoculated with *M. nivale* had fewer tillers and lower dry weight than plants grown from uninoculated seeds. Hare (1997) described how seed-borne *M. nivale* reduces coleoptile length, shoot length and dry weight of seedlings from untreated seeds, compared to guazatine treated seeds at 6 °C. It is not clear if this is solely due to the detrimental effect of the pathogen, or beneficial effects of the fungicide seed treatment on seedling growth. There is no evidence that guazatine has beneficial or deleterious effects on seedlings.

No published work is available for the effect of *M. nivale* infection on the rate of seed imbibition. Similarly, few investigations have examined the effect of *M. nivale* infection on germination. In work by Rennie *et al.* (1990), seed-borne *M. nivale* infection reduced germination in laboratory tests, especially when seeds were subjected to a cold period. The authors reported a negative relationship (R^2 = 0.713) between the incidence of seeds infected with *M. nivale* and percentage germination of 40 winter wheat seedlots in 1987 but not for 50 seedlots in 1988. However, the authors provided no explanation for the relationship, or possible reasons for differences in observations recorded between the two years.

Cristani (1992) investigated the effect of seed-borne *M. nivale* infection on the germination of eight wheat seedlots. More seeds germinated under a 12 h:25 °C: 12 h:15 °C cycle compared to a 6 h:15 °C: 18 h:10 °C cycle. The number of visibly diseased seeds and

seedlings was also increased at the lower temperature cycle. The author proposed that the high temperature cycle had inhibited *M. nivale* growth during germination and early seedling growth. Glasshouse pot trials have suggested that early seedling growth may be critical to plant growth and seedling blight development. Germination of two spring wheat cultivars surface-inoculated with conidia, was poor (61 %) when germinated at temperatures optimum for *F. graminearum* growth (28 °C). Conversely, germination was improved (77 %) at temperatures outside the optimum growth range of *F. graminearum* (8 to 16 °C) (Dickinson, 1923). Similar findings have also been reported for two spring wheat cultivars naturally infected with *F. graminearum* in glasshouse trials (Gilbert *et al.*, 1997). Work by Hare *et al.* (1995) demonstrated a relationship between rate of emergence and final emergence for winter wheat seedlots infected with *M. nivale* in controlled environments.

Investigations with pathogen-free seeds have indirectly supported the hypothesis that *M. nivale* is responsible for reductions in germination and final emergence under low temperatures and/or dry soil moisture regimes. The rate of wheat germination was slowed under these conditions but final germination was unaffected in the absence of *M. nivale* (Hampson & Simpson, 1990; Kamaha & Maguire, 1992), although this appears to be dependent on the cultivars used. Similar effects of temperature and water potential on rate of seedling emergence have also been demonstrated (DeJong & Best, 1979; Khah *et al.*, 1986).

The aims of the work described in this chapter were to investigate (i) the effect of seed-borne *M. nivale* infection on the rate of imbibition, rate of germination and rate of emergence of winter wheat under different temperature and soil moisture regimes and (ii) the effect of *M. nivale* seedling blight severity on the rate of emergence and length of first leaf through a series of controlled environment studies.

MATERIALS & METHODS

The effect of seed-borne *Microdochium nivale* infection on rate of imbibition

An experiment was designed to test the hypothesis that seed-borne *M. nivale* does not slow the rate of wheat seed imbibition more at low temperatures and reduced water potentials. Seedlots 2 (cv. Cadenza; 29 % infection), 5 (cv. Equinox: 45 % infection), 6 (cv. Equinox: 88 % infection), 7 (cv. Hereward: 55 % infection), 8 (cv. Riband: 30 % infection), 10 (cv. Riband; 73 % infection) and 11 (cv. unknown; 36 % infection) (Table 2: page 33) were used to give a range of cultivars and percentage *M. nivale* infections. Forty surface-sterilised seeds from each seedlot were placed crease down on two sheets of autoclaved Whatman no. 1 filter paper in an inverted 9 cm diameter Petri dish. Five ml of sterile distilled water or PEG 4000 solutions equivalent to -0.5, -1.0, -1.5, -2.0 or -3.0 MPa were added and the dish sealed with Parafilm® (Pechiney Plastic Packaging Inc., Neenah, WI 54956, USA). Dishes were incubated in darkness at 15 or 5 °C. Each moisture regime was replicated four times at each temperature.

Dishes were examined at the same time each day and imbibed seeds removed. Seeds were classed as imbibed when the radicle was visible. Removed seeds were rinsed with sterile distilled water, surface-sterilised and plated onto amended PDA according to the procedures outlined in Chapter 3. Dishes were incubated in darkness at 15 °C and seeds determined as *M. nivale* infected or non-infected. Rate of imbibition in each dish was calculated using Khah *et al.* (1986) equation for rate of emergence (Equation 2) for *M. nivale* infected and non-infected seeds. Imbibition was classified as complete when no seeds imbibed within a dish for five consecutive days. All unimbibed seeds were surface-sterilised, plated onto amended PDA and incubated as above. Rate of imbibition was statistically analysed for each seedlot using factorial ANOVA with *M. nivale* infection,

moisture regime and temperature as factors. Data was square root transformed prior to analysis for some seedlots to obtain normally distributed data.

The effect of seed-borne *Microdochium nivale* infection on rate of germination

An experiment was designed to test the hypothesis that seed-borne *M. nivale* does not slow rate of germination more at low temperatures and reduced water potentials. Seedlots 2 (cv. Cadenza; 29 % infection), 5 (cv. Equinox: 45 % infection), 6 (cv. Equinox: 88 % infection), 7 (cv. Hereward; 55 % infection), 8 (cv. Riband; 30 % infection), 10 (cv. Riband; 73 % infection) and 11 (cv. unknown: 36 % infection) (Table 2; page 33) were used to give a range of cultivars and percentage *M. nivale* infections. Twenty-five surface-sterilised seeds from each seedlot were placed crease down on two sheets of autoclaved Whatman no. 1 filter paper in an inverted 9 cm diameter Petri dish. Five ml of sterile distilled water or a PEG 4000 solution equivalent to -1 MPa were added and the dish sealed with Parafilm®. Dishes were incubated in darkness at 15 or 5 °C. Each moisture regime was replicated four times for each temperature.

Dishes were examined at the same time each day and germinated seeds removed. Seeds were classed as having germinated when the coleoptile was longer than 0.5 cm and three roots longer than 0.5 cm, or the total root length greater than 3 cm. Germinated seeds were removed, rinsed, surface-sterilised and plated onto amended PDA according to the procedures outlined in Chapter 3. Dishes were incubated at 15 °C in darkness and seeds were determined as *M. nivale* infected or non-infected. Rate of germination in each dish was calculated using Khah *et al.* (1986) equation for rate of emergence (Equation 2) for *M. nivale* infected and non-infected seeds. After 300 °Cd, all ungerminated seeds were surface-sterilised, plated onto amended PDA and incubated as above.

Rate of germination for each seedlot was analysed statistically using factorial ANOVA with *M. nivale* infection, moisture regime and temperature as factors. Data for some seedlots was either square root or natural logarithm transformed to ensure normally distributed data.

The effect of seed-borne *Microdochium nivale* infection on rate of emergence

An experiment was designed to test the hypothesis that severe seed-borne *M. nivale* does not slow rate of emergence. Seedlots 2 (cv. Cadenza; 29 % infection), 5 (cv. Equinox; 45 % infection), 6 (cv. Equinox; 88 % infection), 7 (cv. Hereward; 55 % infection), 8 (cv. Riband; 30 % infection), 10 (cv. Riband; 73 % infection) and 11 (cv. unknown; 36 % infection) (Table 2; page 33) were used to give a range of cultivars and percentage *M. nivale* infections. Fifty surface-sterilised seeds from each seedlot were planted crease down at 20 mm depth in sterilised John Innes No. 2 compost in seed trays, see Chapter 3; page 33. Trays were placed into a dark controlled environment cabinet (Conviron, Controlled Environments Ltd, Winnipeg, Manitoba, Canada) set at 10 °C. Trays were watered every two days using sterile distilled water. Each seedlot had four replicates.

Trays were examined at the same time each day and emerged seedlings (GS 10) removed. Seeds from emerged seedlings were surface-sterilised and plated onto amended PDA according to the procedures outlined in Chapter 3. Dishes were incubated in darkness at 15 °C and seeds determined as *M. nivale* infected or non-infected. Rate of emergence was calculated (Equation 2) for *M. nivale* infected and non-infected seeds in each tray. After 300 °Cd, all unemerged seeds were surface-sterilised and plated onto amended PDA in darkness at 15 °C. Rate of emergence for infected and non-infected seeds for each seedlot was statistically analysed using a t-test.

The effect of seedling blight disease severity from seed-borne *Microdochium nivale* infection on rate of emergence and first leaf length

An experiment was designed to test the hypothesis that severe seedling blight from seed-borne *M. nivale* does not slow the rate of emergence or adversely affect first leaf length. Seedlots 2 (cv. Cadenza; 29 % infection), 5 (cv. Equinox; 45 % infection), 6 (cv. Equinox; 88 % infection), 7 (cv. Hereward; 55 % infection), 8 (cv. Riband; 30 % infection), 10 (cv. Riband; 73 % infection) and 11 (cv. unknown; 36 % infection) (Table 2; page 33) were used to give a range of cultivars and percentage *M. nivale* infections. Fifty surface-sterilised seeds from each seedlot were planted crease down at 20 mm depth in sterilised John Innes No. 2 compost in seed trays (Chapter 3). Trays were placed into a Conviron set at 10 °C with 12 h light (10 μmol m^{-2} s^{-1} mean photon flux density). Trays were watered every two days using sterile distilled water. Each seedlot had four replicates. Trays were examined at the same time each day and seedling emergence (GS 10) recorded. After 250 °Cd, seedlings (GS 12) were assessed for seedling blight severity (Table 4). First leaf lengths (coleoptile to leaf tip) were also measured. Rate of emergence for each disease score was calculated (Equation 2). Rate of emergence and first leaf length for each seedlot were analysed by ANOVA with seedling blight severity (0, 1, 2, 3 and 4) as factors.

RESULTS

The effect of seed-borne *Microdochium nivale* infection on rate of imbibition

Microdochium nivale was frequently isolated from unimbibed seeds. For all seedlots, rate of imbibition was significantly slower ($P < 0.05$) at 5 than 15 °C. For all seedlots, rate of imbibition was significantly slower ($P < 0.05$) with each reduction in water potential (Table 6). Rate of imbibition was significantly faster ($P < 0.05$) at 15 than 5 °C across all water potentials except for -3 MPa. There was no consistent effect of *M. nivale* infection on rate of imbibition across all seedlots. However, seeds of *M. nivale* infected seeds

imbibed significantly slower ($P < 0.05$) than pathogen-free seeds for seedlots 2 (infected seeds 0.2200 day^{-1}; pathogen-free seeds 0.2322 day^{-1}), 7 (infected seeds 0.1808 day^{-1}; pathogen-free seeds 0.1968 day^{-1}), 8 (infected seeds 0.1819 day^{-1}; pathogen-free seeds 0.2130 day^{-1}), 10 (infected seeds 0.2444 day^{-1}; pathogen-free seeds 0.2330 day^{-1}) and 11 (infected seeds 0.1766 day^{-1}; pathogen-free seeds 0.2075 day^{-1}).

The seed-borne infection x temperature interaction was only significant for three of the seven seedlots tested. Infected seeds of seedlot 7 had slightly faster rate of imbibition at 15 °C (0.2783 day^{-1}) than pathogen-free seeds (0.2651 day^{-1}) but infected seeds (0.0833 day^{-1}) had significantly slower ($P < 0.05$) rate of imbibition than pathogen-free seeds (0.1285 day^{-1}) at 5 °C. Infected seeds (0.1050 day^{-1}) of seedlot 8 imbibed significantly slower ($P < 0.05$) than pathogen-free seeds (0.1367 day^{-1}) only at 5 °C. Infected seeds from seedlot 10 had significantly faster ($P < 0.05$) rate of imbibition (0.3438 day^{-1}) than pathogen-free seeds (0.3176 day^{-1}) only at 15 °C

A significant interaction between seed-borne *M. nivale* infection x water potential only occurred for seedlots 2 ($P = 0.034$), 7 ($P < 0.001$) and 8 ($P < 0.001$). However, this interaction was inconsistent. Rate of imbibition of infected seeds of seedlot 2 was significantly faster than pathogen-free seeds only at 0, -0.5 and -1 MPa ($P < 0.05$). Rate of imbibition for infected seeds (0.2065 day^{-1}) of seedlot 7 was significantly ($P < 0.05$) below pathogen-free seeds (0.3263 day^{-1}) only at 0 MPa. Rate of imbibition for infected and pathogen-free seeds then declined with reductions in water potential. Rate of imbibition for infected and pathogen-free seeds of seedlot 8 was significantly slower ($P < 0.05$) with reductions in water potential.

The seed-borne *M. nivale* infection x temperature x water potential interaction was only significant for seedlots 7 ($P = 0.005$) and 8 ($P < 0.001$). For both seedlots, rate of

Table 6. Effect of water potential and temperature on rate of imbibition (day^{-1}) for seven winter wheat seedlots infected with *Microdochium nivale* in controlled environments.

			rate of imbibition (day^{-1}) water potential (MPa)					
seedlot	**temp (°C)**	**infection[1]**	**0**	**-0.5**	**-1.0**	**-1.5**	**-2.0**	**-3.0**
2	15	Y	0.590	0.429	0.346	0.274	0.187	0.000
	15	N	0.679	0.399	0.368	0.290	0.202	0.000
	5	Y	0.189	0.187	0.153	0.131	0.103	0.051
	5	N	0.202	0.187	0.167	0.135	0.105	0.053
2*	15	Y	0.767	0.655	0.588	0.523	0.434	0.000
	15	N	0.823	0.631	0.606	0.538	0.448	0.000
	5	Y	0.434	0.433	0.391	0.361	0.321	0.225
	5	N	0.450	0.432	0.409	0.368	0.324	0.229
LSD ($P < 0.05$) = 0.0322, SED = 0.0161, DF = 72, %cv = 5.3								
5	15	Y	0.696	0.497	0.420	0.339	0.284	0.101
	15	N	0.706	0.484	0.426	0.349	0.279	0.090
	5	Y	0.248	0.186	0.161	0.140	0.139	0.079
	5	N	0.255	0.197	0.164	0.147	0.131	0.081
5*	15	Y	0.834	0.705	0.647	0.582	0.532	0.317
	15	N	0.840	0.695	0.652	0.591	0.526	0.259
	5	Y	0.498	0.431	0.401	0.374	0.372	0.281
	5	N	0.505	0.444	0.404	0.383	0.362	0.285
LSD ($P < 0.05$) = 0.0597, SED = 0.0300, DF = 72, %cv = 8.5								
6	15	Y	0.574	0.419	0.379	0.323	0.257	0.104
	15	N	0.596	0.469	0.392	0.278	0.243	0.064
	5	Y	0.201	0.179	0.159	0.131	0.131	0.072
	5	N	0.218	0.179	0.166	0.145	0.135	0.073
6*	15	Y	0.757	0.647	0.615	0.568	0.507	0.322
	15	N	0.771	0.685	0.626	0.527	0.492	0.219
	5	Y	0.448	0.422	0.398	0.360	0.362	0.269
	5	N	0.466	0.423	0.407	0.380	0.367	0.269
LSD ($P < 0.05$) = 0.0535, SED = 0.0268, DF = 72, %cv = 8.1								
7	15	Y	0.413	0.394	0.353	0.275	0.177	0.060
	15	N	0.443	0.346	0.332	0.233	0.162	0.075
	5	Y	0.185	0.115	0.112	0.102	0.101	0.070
	5	N	0.210	0.132	0.130	0.111	0.106	0.083
LSD ($P < 0.05$) = 0.0473, SED = 0.0237, DF = 72, %cv = 17.8								

* data square root transformed.

1 **Y** seeds infected with *M. nivale*; **N** seeds not infected with *M. nivale*.

Seedlot **2** (cv. Cadenza; 29 % infection), **5** (cv. Equinox; 45 % infection), **6** (cv. Equinox; 88 % infection), **7** (cv. Hereward; 55 % infection) see Table 2 page 33.

Table 6. continued.

seedlot	temp (°C)	infection[1]	rate of imbibition (day^{-1}) water potential (MPa) 0	-0.5	-1.0	-1.5	-2.0	-3.0
8	15	Y	0.496	0.342	0.323	0.282	0.184	0.086
	15	N	0.482	0.370	0.323	0.281	0.213	0.067
	5	Y	0.162	0.113	0.091	0.121	0.069	0.074
	5	N	0.194	0.150	0.133	0.132	0.124	0.087
LSD ($P < 0.05$) = 0.0358, SED = 0.0180, DF = 72, %cv = 12.9								
10	15	Y	0.493	0.432	0.420	0.332	0.279	0.108
	15	N	0.453	0.413	0.395	0.291	0.251	0.103
	5	Y	0.246	0.153	0.136	0.128	0.112	0.094
	5	N	0.205	0.172	0.161	0.135	0.120	0.098
LSD ($P < 0.05$) = 0.0300, SED = 0.0150, DF = 72, %cv = 8.9								
11	15	Y	0.424	0.345	0.282	0.223	0.182	0.096
	15	N	0.441	0.375	0.313	0.272	0.194	0.089
	5	Y	0.094	0.108	0.112	0.093	0.094	0.067
	5	N	0.211	0.157	0.133	0.116	0.110	0.081
LSD ($P < 0.05$) = 0.0503, SED = 0.0252, DF = 72, %cv = 18.6								

1 **Y** – seeds infected with *M. nivale*; **N** – seeds not infected with *M. nivale*.
Seedlot **8** (cv. Riband; 30 % infection), **10** (cv. Riband; 73 % infection), **11** (cv. unknown; 36 % infection), see Table 2; page 33.

imbibition was significantly slower ($P < 0.05$) than pathogen-free seeds only at 5 °C at 0 MPa.

The effect of seed-borne *Microdochium nivale* infection on rate of germination

Microdochium nivale was frequently isolated from ungerminated seeds. Rate of germination for all seven seedlots was significantly faster ($P < 0.05$) at 15 than 5 °C. Reduced water potential significantly slowed ($P < 0.05$) rate of germination for all seedlots except seedlot 10. Rate of germination was significantly slower ($P < 0.05$) at -1 than 0 MP for all seven seedlots tested (Table 7).

The temperature x water potential interaction was significant for six of the seven seedlots ($P < 0.05$). Rate of germination for seeds of seedlot 2, 5, 7 and 11 grown at 15 °C was

Table 7. Effect of water potential and temperature on rate of germination (day^{-1}) for seven winter wheat seedlots infected with *Microdochium nivale* in controlled environments.

			rate of germination (day^{-1}) water potential (MPa)	
seedlot	temp (°C)	infection[1]	0	-1
2	15	Y	0.216	0.165
	15	N	0.287	0.195
	5	Y	0.064	0.068
	5	N	0.080	0.076
2*	15	Y	0.462	0.406
	15	N	0.536	0.441
	5	Y	0.253	0.259
	5	N	0.282	0.276
LSD ($P < 0.05$) = 0.0375, SED = 0.0182, DF = 24, %cv = 7.1				
5	15	Y	0.298	0.226
	15	N	0.299	0.213
	5	Y	0.075	0.060
	5	N	0.087	0.065
5*	15	Y	0.545	0.475
	15	N	0.546	0.461
	5	Y	0.273	0.245
	5	N	0.294	0.254
LSD ($P < 0.05$) = 0.0200, SED = 0.0097, DF = 24, %cv = 3.5				
6	15	Y	0.269	0.186
	15	N	0.256	0.186
	5	Y	0.066	0.045
	5	N	0.057	0.048
6*	15	Y	0.518	0.431
	15	N	0.504	0.431
	5	Y	0.257	0.212
	5	N	0.238	0.219
LSD ($P < 0.05$) = 0.0256, SED = 0.0175, DF = 24, %cv = 7.0				
7	15	Y	0.176	0.151
	15	N	0.173	0.139
	5	Y	0.029	0.035
	5	N	0.039	0.044
LSD ($P < 0.05$) = 0.0240, SED = 0.0117, DF = 24, %cv = 16.8				

* data square root transformed.

1 **Y** - seeds infected with *M. nivale*; **N** - seeds not infected with *M. nivale*.

Seedlot **2** (cv. Cadenza; 29 % infection), **5** (cv. Equinox; 45 % infection), **6** (cv. Equinox; 88 % infection), **7** (cv. Hereward; 55 % infection) see Table 2; page 33.

Table 7. continued.

			rate of germination (day^{-1}) water potential (MPa)	
seedlot	**temp (°C)**	**infection**	**0**	**-1**
8	15	Y	0.165	0.106
	15	N	0.185	0.130
	5	Y	0.040	0.046
	5	N	0.062	0.063
8**	15	Y	-1.82	-2.25
	15	N	-1.72	-2.05
	5	Y	-3.22	-3.11
	5	N	-2.78	-2.76
LSD ($P < 0.05$) = 0.177, SED = 0.122, DF = 24, %cv = 7.0				
10	15	Y	0.207	0.190
	15	N	0.171	0.163
	5	Y	0.080	0.064
	5	N	0.071	0.058
10*	15	Y	0.452	0.435
	15	N	0.411	0.402
	5	Y	0.282	0.253
	5	N	0.266	0.240
LSD ($P < 0.05$) = 0.0446, SED = 0.0216, DF = 24, %cv = 8.9				
11	15	Y	0.114	0.147
	15	N	0.150	0.153
	5	Y	0.046	0.037
	5	N	0.018	0.045
11**	15	Y	-2.19	-1.92
	15	N	-1.94	-1.88
	5	Y	-2.81	-3.30
	5	N	-2.64	-3.10
LSD ($P < 0.05$) = 0.275, SED = 0.132, DF = 24, %cv = 7.5				

* data square root transformed.

** data natural logarithm transformed.

1 **Y** – seeds infected with *M. nivale*; **N** – seeds not infected with *M. nivale*.

Seedlot **8** (cv. Riband; 30 % infection), **10** (cv. Riband; 73 % infection) **11** (cv. unknown; 36 % infection) see Table 2; page 33.

significantly quicker than seeds grown at 5 °C at both water potentials ($P < 0.05$). Rate of germination for seedlot 8 at 15 °C was significantly slower at -1 MPa (0.118 day^{-1}) compared to 0 MPa (0.1746 day^{-1}). There was no significant reduction in rate of

germination at 5 °C. Rate of germination of seedlots 5 and 8 at 15 °C was significantly slower at -1 MPa compared to 0 MPa ($P < 0.05$). However, there was no significant reduction in rate of germination at 5 °C for either seedlot.

Seed-borne *M. nivale* infection significantly slowed ($P < 0.05$) rate of germination of seedlots 2 (pathogen-free seeds 0.1598 day^{-1}; infected seeds 0.1281 day^{-1}), 8 (pathogen-free seeds 0.1101 day^{-1}; infected seeds 0.0891 day^{-1}) and 11 (pathogen-free seeds 0.0913 day^{-1}; infected seeds 0.0861 day^{-1}). The opposite trend occurred for seedlot 10 (infected seeds 0.1349 day^{-1}; pathogen-free seeds 0.1156 day^{-1}).

The seed-borne *M. nivale* infection x temperature interaction was only significant for seedlots 5 and 8 ($P < 0.05$). Pathogen-free seeds of seedlot 5 germinated significantly faster (0.2742 day^{-1}) at 5 °C than infected seeds (0.2588 day^{-1}). Pathogen-free seeds of seedlot 8 had significantly faster rate of germination than infected seeds at 15 °C (pathogen-free seeds 0.1573 day^{-1}; infected seeds 0.1353 day^{-1}) and 5 °C (pathogen-free seeds 0.0629 day^{-1}; infected seeds 0.0429 day^{-1}). The seed-borne *M. nivale* infection x water potential interaction and the seed-borne *M. nivale* infection x temperature x water potential interaction was not significant for any of the seven seedlots.

The effect of seed-borne *Microdochium nivale* infection on rate of emergence

Diseased seedlings were produced from all seedlots. *Microdochium nivale* was frequently isolated from ungerminated seeds and seedlings that had germinated but not emerged. Rate of emergence was dependent on the seedlot (Table 8). Seedlings from *M. nivale* infected seeds emerged faster than seedlings from pathogen-free seeds in seedlots 6, 8, 10 and 11 but only for seedlots 6 and 10 was the effect significant ($P < 0.05$). The opposite but not significant trend was seen for seedlots 2, 5 and 7.

Table 8. Effect of seed-borne *Microdochium nivale* infection on rate of seedling emergence (day^{-1}) for seven winter wheat seedlots at 10 °C.

	rate of emergence (day^{-1})		
seedlot	***M. nivale* infected seeds**	***M. nivale* uninfected seeds**	**probability***
2	0.08190	0.08573	0.458
5	0.08725	0.09078	0.155
6	0.07390	0.06615	0.050
7	0.05812	0.06218	0.320
8	0.06455	0.05815	0.327
10	0.07910	0.06680	0.035
11	0.07260	0.06785	0.276

* t-test analysis.
Seedlots **2** (cv. Cadenza; 29 % infection), **5** (cv. Equinox; 45 % infection), **6** (cv. Equinox; 88 % infection), **7** (cv. Hereward; 55 % infection), **8** (cv. Riband; 30 % infection), **10** (cv. Riband; 73 % infection), **11** (cv. unknown; 36 % infection) Table 2; page 33.

The effect of seedling blight disease severity from seed-borne *Microdochium nivale* infection on rate of emergence and first leaf length

Diseased seedlings were produced by all seedlots. Differences were observed in seedling blight disease severity. For all seedlots, severely diseased seedlings (disease scores 3 and 4) emerged slower than healthy or slightly diseased seedlings (disease scores 0, 1 and 2). However, these differences were only significant for seedlots 2, 5, and 6 (Table 9). *Microdochium nivale* disease severity from seed-borne inoculum significantly reduced first leaf length ($P < 0.05$) for all seven seedlots. First leaf length significantly decreased ($P < 0.05$) with increased disease severity for all seedlots (Table 10).

DISCUSSION

Seed-borne *M. nivale* infection had no consistent effect on the early stages of winter wheat seedling growth and development under the experimental conditions used. Only for seedlot 2 (cv. Cadenza; 29 % infection) did seed-borne *M. nivale* infection slow rate of imbibition, germination and emergence. Hare (1997) reported good linear relationships between disease severity and rate of seedling emergence for winter wheat seedlots surface-

Table 9. Effect of *Microdochium nivale* disease severity at GS 12 from seed-borne infection on rate of seedling emergence (day^{-1}) for seven winter wheat seedlots grown at 10 °C.

	rate of emergence (day^{-1})						
	seedlot						
disease score	**2**	**5**	**6**	**7**	**8**	**10**	**11**
0	0.071	0.107	0.071	0.084	0.099	0.089	0.103
1	0.074	0.102	0.072	0.089	0.092	0.084	0.092
2	0.072	0.098	0.079	0.069	0.082	0.060	0.081
3	0.061	0.074	0.066	*	*	*	0.081
4	0.049	*	0.054	*	*	*	*
LSD ($P < 0.05$)	0.0157	0.0220	0.0097	NS	NS	0.0221	NS
SED	0.0068	0.0097	0.0044	0.0096	0.0083	0.0090	0.0100
DF	19	15	19	11	11	11	15
%cv	14.7	14.4	9.0	16.8	12.9	16.4	15.9

* no seedlings in that category.
Seedlots **2** (cv. Cadenza; 29 % infection), **5** (cv. Equinox; 45 % infection), **6** (cv. Equinox; 88 % infection), **7** (cv. Hereward; 55 % infection), **8** (cv. Riband; 30 % infection), **10** (cv. Riband; 73 % infection), **11** (cv. unknown; 36 % infection) see Table 2; page 33.

Table 10. Effect of *Microdochium nivale* disease severity at GS 12 from seed-borne infection on first leaf length (mm) for seven winter wheat seedlots grown at 10 °C.

	length of first leaf (mm)						
	seedlot						
disease score	**2**	**5**	**6**	**7**	**8**	**10**	**11**
0	111	99	90	85	110	97	101
1	100	88	88	79	104	96	96
2	92	87	78	28	71	59	65
3	56	39	63	*	*	*	25
4	28	*	30	*	*	*	*
LSD ($P < 0.05$)	25.5	19.1	20.0	26.7	32.3	29.7	14.2
SED	11.0	8.5	9.0	10.4	13.2	12.2	6.3
DF	19	15	19	11	11	11	15
%cv	20.1	15.3	18.2	22.9	19.6	20.4	12.4

* no seedlings in that category.
Seedlots **2** (cv. Cadenza; 29 % infection), **5** (cv. Equinox; 45 % infection), **6** (cv. Equinox; 88 % infection), **7** (cv. Hereward; 55 % infection), **8** (cv. Riband; 30 % infection), **10** (cv. Riband; 73 % infection), **11** (cv. unknown; 36 % infection) see Table 2; page 33.

inoculated and naturally infected with *F. culmorum* and *M. nivale* respectively. This implies that slower rate of emergence increases the opportunity for transfer of seed-borne infection to the seedling. The trend across all seedlots tested here, that more heavily diseased seedlings showed slowed emergence, although seed infection only significantly slowed the rate of emergence below rate of emergence from pathogen-free seeds for one seedlot, appears to support this hypothesis.

Indirect evidence exists to suggest that faster rate of emergence reduces the opportunity for infection. Winter wheat (cv. Viking) seeds soaked in water for 1 to 12 h before *F. culmorum* inoculation and planting had faster rate of seeding emergence and increased root growth (Malalasekera & Colhoun, 1968). Pre-emergent death and disease severity was also reduced in comparison to unsoaked seeds. Seedlings from seeds soaked for three days had increased disease severity. This supports the hypothesis that depressed seedling vigour, increases the opportunity for infection. However, in subsequent work by Fukui *et al.* (1994), soaking wheat seeds prior to sowing decreased the incidence of embryo infection from soil-borne *Pythium ultimum* inoculum. They concluded this was due to increased loss of seed exudate during soaking and not the effect of soaking on rate of seedling emergence.

Seed-borne *M. nivale* infection only significantly slowed emergence (GS 10) for seedlot 10 (cv. Riband; 73 % infection) but faster rate of emergence occurred from diseased seeds of seedlots 6, 8, 10 and 11. However, severely diseased seedlings (GS 12) did emerge more slowly than less severely diseased or healthy seedlings. In addition, the more severe the seedling blight severity, the greater the reduction in first leaf length. This may reduce the chances of seedling recovery, reducing establishment numbers and lower subsequent plant productivity. This will be addressed further in Chapter 7.

It is possible that seeds giving rise to heavily diseased seedlings contained more seed-borne inoculum or inoculum that was situated in a more critical location within the seed. *Microdochium nivale* may be present in all cereal seed components but is most abundant in the inner pericarp/outer epidermis (Bateman, 1983; Chelkowski *et al.*, 1990; Cristani, 1992). Bateman proposed that inoculum in these seed components was predominantly responsible for causing seedling blight. *Microdochium nivale* might only be expected to slow seedling growth once the disease has manifested itself on the coleoptile and can directly affect seedling growth. Seeds were screened before use to ensure that reasonably constant seed sizes were used. Different seed sizes may possess different amounts of fungal inoculum. This has not been investigated but may influence seedling blight severity. Hare *et al.* (1999) demonstrated that the distribution of *M. nivale* infection within seeds of three winter wheat (cv. Riband) seedlots was similar to that of the seed weight distribution within the seedlots.

Microdochium nivale infected seeds had significantly slower imbibition for five of the seven seedlots tested. That *M. nivale* was frequently isolated from unimbibed seeds implies that it is capable of killing seeds before or during imbibition, although it was impossible to determine the exact timing of seed death. Appearance of the radicle through the seed coat was found to be the most convenient and accurate measure for determining completion of seed imbibition. Germination of seeds infected with *M. nivale* was only significantly faster than pathogen-free seeds for four seedlots. Germination studies were conducted for 300 °Cd to give the seeds maximum opportunity for germination. Disease severity was measured after 250 °Cd. This period afforded all seedlings time to emerge and was sufficient for disease development. Millar & Colhoun (1969a) recorded that pre-emergent death of winter wheat (cv. Viking) from seeds surface-inoculated with *M. nivale* usually occurred before germination was complete. Work by Manka & Chelkowski (1985) suggested that *M. nivale* has a detrimental effect on germinating seeds. Culture filtrates of

twelve *M. nivale* isolates exhibited phytotoxicity towards wheat seeds and inhibited germination and seedling growth at 20 °C. Enniatins (non-host specific phytotoxins) produced by *F. tricinctum* reduced seminal root and primary leaf growth of wheat (cv. Arthur) in Petri dishes (Burmeister & Plattner, 1987). It is likely that *M. nivale* infection within the seeds as mycelium or culture filtrate applied to the seed-surface will have different effects on germinating wheat seeds.

Fungal invasion into the seed during anthesis, grain fill and ripening can affect aspects of early seedling growth. Severity of *F. graminearum* infection has been related to grain damage. Lightly infected hard red wheat grains had numerous hypha in the sub-aleurone region, caryopsis coat and aleurone cells. Heavily infected grains had lost most of their structure, including the aleurone layer, starch granules, storage proteins and cell walls (Bechtel *et al.*, 1985). Boyacioglu & Hettiarachchy (1995) described hard red spring wheat (cv. Len) seeds lightly and moderately infected with *F. graminearum* having different biochemical compositions. Moderately infected seeds had increased reducing sugars and lipids, whilst lightly infected seeds had increased protein, total sugars, non-starch and starch lipid levels. Cellulose and hemicellulose levels were reduced in both infected grain types, whilst albumin and glutenin proteins were reduced only in heavily infected seeds. It is likely that heavily infected grains will have reduced vigour, due to the loss of storage substances and cellular structures required for growth. *Microdochium nivale* is not considered as aggressive a pathogen as *F. graminearum* (Chelkowski *et al.*, 1990). Therefore, it is unlikely that such extreme effects on early seedling growth would be observed for seed-borne *M. nivale* infection.

Differences in cultivars, and an interaction with the experimental conditions used may also have influenced the relationship between seed-borne *M. nivale* and early seedling growth. Differences in winter wheat cultivar susceptibility to *Fusarium* seedling blight have been

reported for seed surface-borne inoculum (Arseniuk *et al.*, 1993) and soil-borne inoculum (Pavlova & Srobarova, 1997) in glasshouse trials. Temperature, water potential and the temperature x water potential interaction were important factors affecting rate of seed imbibition and germination for all seedlots in this investigation. Ashraf & Abu-Shakra (1978), Lafond & Baker (1986), Hampson & Simpson (1990), Kamaha & Maguire (1992) and Wuest *et al.* (1999) have all reported similar findings for pathogen-free wheat cultivars. Rate of seed water uptake has been shown to be slowed at lower temperatures (<10 °C) (Chaudhary *et al.*, 1971: Addae & Pearson, 1992) and dry soils (Dasberg, 1971). Sub-optimal conditions (low temperatures and moisture stresses) slow seedling growth by reducing water uptake but seed and seedling water contents for growth remain unchanged. Therefore, the observation by Ramakrishna *et al.* (1993) that *F. poae*, a weak seedling blight pathogen, grew quickly at 0.97 *aw* (available water) and 0.95 *aw* but more slowly at 0.90 *aw* on irradiated grain is probably of no relevance in seedling blight epidemiology.

CHAPTER 5

Relationship between *Microdochium nivale* growth and seedling growth under different temperature and soil water regimes

INTRODUCTION

Microdochium nivale seedling blight is most severe in cold dry soils (Hare, 1997), conditions which also delay all phases of seedling growth. Rate of water uptake but not final amounts of water in pathogen-free wheat seeds (cv. Thell) has been shown to be dependent on soil water content (Dasberg, 1971) and temperature (Chaudhary *et al.*, 1971). Temperature and osmotic stress have been reported to have accentuating effects in reducing rate of germination of pathogen-free wheat seeds but final germination was typically unaffected (Ashraf & Abu-Shakra, 1978; Lafond & Baker, 1986; Hampson & Simpson, 1990; Kamaha & Maguire, 1992). Investigations have shown similar temperature and soil water content effects on rate of winter wheat seedling emergence and final emergence (Lindstrom *et al.*, 1976; DeJong & Best, 1979; Khah *et al.*, 1986). These published data appear to imply that slowest seedling growth occurs at low temperatures and dry soils. Under these conditions, *M. nivale* seedling blight increases, possibly due to increased opportunity for infection. That *M. nivale* could be isolated from mesocotyls and roots of three oat cultivars grown from pathogen-free seeds, six months after an October but not an April sowing (Rawlinson & Colhoun, 1969), suggests this to be the case.

However, in all investigations, the interaction between seed-borne *M. nivale* infection and winter wheat seedling growth and subsequent seedling blight development remains unquantified. Advances in PCR technologies offer an opportunity to further investigate this complex host-pathogen system and possible differences between the two *M. nivale* sub-species. The aims of this work were to: (i) determine growth rates of *M. nivale* var. *majus* and var. *nivale in vitro*. (ii) investigate the effect of duration of imbibition on fungus biomass within seeds and subsequent seedling growth. (iii) examine the effect of temperature and soil water on the interaction between seedling growth and *M. nivale* var.

majus and var. *nivale* fungus biomass. These aims were achieved through a series of controlled environment investigations.

MATERIALS & METHODS

Effect of temperature on *in vitro Microdochium nivale* growth

An experiment was designed to test the hypothesis that there is no difference between *M. nivale* var. *majus* and var. *nivale* growth rates on agar between 5 and 20 °C. Ten *M. nivale* isolates (Table 11) were cultured on PDA at 15 °C for 8 days. Five mm plugs from the edges of actively growing colonies were transferred to Petri dishes containing 20 ml wheat flour agar (5 % w/w wheat flour: 2 % w/w No. 1 agar (Oxoid Ltd. Basingstoke, UK)) and sealed with Parafilm®. Four dishes for each isolate were incubated in darkness at 5, 10, 15 and 20 °C. Fungus colony diameters were measured in two directions at 90 ° angles at two day intervals until plates became overgrown. Fungus growth rates (mm day^{-1}) were calculated for each temperature and used to determine isolate growth rates between 5 and 20 °C. Base temperatures were calculated by extrapolation following simple regression of each isolates growth rate. Growth rates and base temperatures for growth of *M. nivale* var. *majus* and var. *nivale* were statistically tested using t-test analysis.

Relationship between *in vitro* growth rate of *Microdochium nivale* sub-species and rate of imbibition, rate of emergence and seedling fresh weight increase of winter wheat

An experiment was designed to test the hypothesis that fungus:seedling growth rate ratios at GS 01 and GS 10 for *M. nivale* var. *majus* and var. *nivale* at three temperatures and two soil moisture contents cannot explain reported differences in sub-species distributions in winter wheat. One hundred surface-sterilised seeds of seedlot 1 (cv. Cadenza 0 % infection) (Table 2: page 33) of known weight were placed on to sterile blotting paper in

Table 11. *Microdochium nivale* isolates used for the *in vitro* growth trial.

isolate	sub-species*
2/2/M	*majus*
24/3/M	*majus*
47/2/M	*majus*
53/2/M	*majus*
S048/2/M	*majus*
2/1/N	*nivale*
36/1/N	*nivale*
68/2/N	*nivale*
74/1/N	*nivale*
117/1/N	*nivale*

* determined by PCR method of Nicholson *et al.* (1996).

175 x 115 x 60 mm boxes (Azpack Ltd, Loughborough, UK). Twenty ml of sterile distilled water or a PEG 4000 solution equivalent to -1.5 MPa was added. Boxes were incubated in darkness at 5, 10 or 15 °C. Each treatment was replicated four times. Boxes were examined at the same time each day and imbibed seeds (GS 01; radicle visible) removed, rinsed in sterile distilled water, blotted dry and weighed. Imbibition was classed as complete when no seeds imbibed within a box on five consecutive days.

One hundred surface-sterilised seeds of known weight of seedlot 1 were planted 20 mm deep in autoclaved John Innes No. 2 compost at 15 % w/w soil water content in trays (Table 3). Eight trays were incubated at 5, 10 or 15 °C. Four trays were heavily watered and four trays maintained at 15 % w/w soil water content every five days using sterile distilled water. Trays were examined at the same time every day and emerged seedlings (GS 10) carefully removed, rinsed in sterile distilled water, blotted dry and weighed. Emergence was classed as complete when no seedlings emerged on five consecutive days.

Percentage increase in seedling fresh weight at GS 01 and GS 10 was calculated using Equation 5. *In vitro* growth rates of *M. nivale* var. *majus* and var. *nivale* were used to calculate fungus:seedling growth rate ratios at GS 01 and GS 10 at 5, 10 and 15 °C

(Equation 6). Fungus:seedling growth rate ratios at GS 01 and GS 10 were compared using factorial ANOVA with temperature, soil water content or water potential and *M. nivale* sub-species as factors. Where appropriate data was square root or natural logarithm transformed to ensure normally distributed data.

$$\% \text{ seedling fresh weight increase} = \frac{\text{seedling weight} - \text{seed weight}}{\text{seed weight}} \times 100 \quad \textbf{Equation 5}$$

$$\text{fungus:seedling growth rate ratio} = \frac{\textit{in vitro} \text{ fungus growth rate}}{\text{increase in seedling fresh weight}} \quad \textbf{Equation 6}$$

Effect of delayed imbibition on amount of *Microdochium nivale* DNA in winter wheat seeds

An experiment was designed to test the hypothesis that delayed imbibition does not increase the amount of *M. nivale* DNA in naturally infected seedlots. Seedlots 2 (cv. Cadenza; 29 % infection) and 6 (cv. Equinox; 88 % infection) (Table 2; page 33) were used in this investigation to provide a range of infection levels and cultivars for comparisons. Forty surface-sterilised seeds of known weight of each seedlot were placed on to two autoclaved sheets of Whatman No.1 filter paper in 24 inverted Petri dishes. Twenty ml of PEG 4000 solution equivalent to -4 MPa was added, dishes sealed with Parafilm[R] and incubated in darkness at 5 °C. After 10, 20, 30, 40 and 50 days, four dishes were removed, seeds rinsed in sterile water, blotted dry, weighed and frozen at -15 °C. The quantity of *M. nivale* DNA in seeds was determined using quantitative PCR (Chapter 3). Four lots of forty seeds were frozen at the onset of the experiment to provide initial quantities of *M. nivale* DNA. Percentage seedling fresh weight increase was calculated using Equation 5.

Effect of delayed imbibition on seedling growth and seedling blight severity from *Microdochium nivale* infected and pathogen-free winter wheat seedlots

An experiment was designed to test the hypothesis that the amount of *M. nivale* DNA in winter wheat seeds has no effect on rate of emergence, final emergence and seedling blight severity. Seedlots 3 (cv. Equinox: 0 % infection) and 6 (cv. Equinox: 88 % infection) (Table 2; page 33) were used in this investigation to provide a range of infection levels for comparisons. Forty surface-sterilised seeds of each seedlot were placed on to two autoclaved sheets of Whatman No.1 filter paper in 24 inverted Petri dishes. Twenty ml of PEG 4000 solution equivalent to -4 MPa was added, dishes sealed with Parafilm® and incubated in darkness at 5 °C. The experiment was designed so that seeds soaked for 0, 30 or 50 days could be planted on the same day. One hundred seeds of each imbibition period were planted 20 mm deep into autoclaved John Innes No. 2 compost in seed trays (Chapter 3). Each treatment had four replicates. Trays were maintained in a moist state in an unheated polythene tunnel and the median temperature recorded (Chapter 3). Seedling emergence was recorded at the same time daily and rate of emergence calculated (Equation 2). At GS 12, seedlings were harvested and final emergence, disease index (Table 4) and seedling dry weight (Chapter 3) determined. Final emergence and seedling dry weight were factorially ANOVA analysed using seedlot and duration of imbibition time as factors. Certain data was square root or natural logarithm transformed prior to analysis to ensure normally distributed data. Rate of emergence and disease index data were not normally distributed and not analysed.

Effect of temperature and soil water content on amount of *Microdochium nivale* DNA and seedling growth from untreated diseased winter wheat seedlots

An experiment was designed to test the hypothesis that temperature and soil water content have no effect on the amount of *M. nivale* DNA in seeds at GS 01 and seedlings at GS 10 in naturally infected seeds. Seedlots 2 (cv. Cadenza; 29 % infection) and 6 (cv. Equinox:

88 % infection) (Table 2; page 33) were used in this investigation to provide a range of infection levels and cultivars for comparisons. One hundred surface-sterilised seeds of known weight of each seedlot were placed on to sterile blotting paper in boxes (175 mm x 115 mm x 60 mm). Twenty ml of sterile distilled water or a PEG 4000 solution equivalent to -1.5 MPa was added and boxes incubated in darkness at 5, 10 and 15 °C. Each treatment had four replicates. Boxes were examined at the same time daily and imbibed (GS 01) seeds removed, rinsed in sterile distilled water, blotted dry, weighed and frozen at -15 °C. Imbibition was classed as complete when no seeds imbibed within a box on five consecutive days. Rate of imbibition was calculated (Equation 2). One hundred seeds were frozen without being imbibed to determine initial amounts of *M. nivale* DNA in seeds (control).

One hundred surface-sterilised seeds of known weight of each seedlot were planted 20 mm deep in autoclaved John Innes No. 2 at 15 % w/w soil water content in trays (Table 3). Eight trays of each seedlot were incubated at 5, 10 and 15 °C. Four trays were heavily watered and four trays maintained at 15 % w/w soil water content, every five days using sterile distilled water. Trays were examined at the same time each day for emerged seedlings. Emerged seedlings (GS 10) were carefully removed, rinsed in sterile distilled water, blotted dry, weighed and frozen at -15 °C. Emergence was classed as complete when no seedlings emerged on five consecutive days. Rate of emergence was calculated using Equation 2. One hundred seeds were frozen without being planted to determine initial amounts of *M. nivale* DNA in seeds (control).

Percentage increase in seedling fresh weight at GS 01 and GS 10 was calculated using Equation 5. *Microdochium nivale* DNA amounts in imbibed seeds (GS 01) and emerged seedlings (GS 10) was determined using quantitative PCR (Chapter 3). Percentage *M.*

nivale DNA in comparison to ungrown seeds was calculated (Equation 7). Fungal DNA:seedling fresh weight ratios at GS 01 and GS 10 were calculated (Equation 8).

$$\% \text{ fungal DNA increase} = \frac{(x - y)}{y} \times 100 \qquad \textbf{Equation 7}$$

where x = fungal DNA after seedling growth; y = fungal DNA in control seeds.

$$\text{fungal DNA:seedling fresh weight ratio} = \frac{x}{y} \qquad \textbf{Equation 8}$$

where x = % fungal DNA increase (Equation 7) and y = % seedling fresh weight increase (Equation 5).

Rate of imbibition and final imbibition, rate of emergence and final emergence and the fungal DNA:seedling fresh weight ratios were ANOVA analysed for each seedlot using temperature and water potential or soil water content as factors. Where appropriate, data were angular or natural logarithm transformed to ensure normally distributed data. Rate of emergence data for seedlot 6 was not normally distributed and not analysed.

RESULTS

Effect of temperature on *in vitro Microdochium nivale* growth

Positive correlations were found between growth rate and temperature for all isolates. Growth rates of all *M. nivale* var. *majus* isolates were similar. Growth rates of the *M. nivale* var. *nivale* isolates were also similar (Figure 2). *Microdochium nivale* var. *majus* isolates (0.79 mm day^{-1}) had a significantly faster growth rate ($P < 0.05$) than var. *nivale* isolates (0.59 mm day^{-1}). More variation was observed in base temperatures for growth than growth rates between and within sub-species. *Microdochium nivale* var. *nivale* isolates had a significantly ($P < 0.05$) higher base temperature for growth (3.6 °C) than var. *majus* isolates (1.7 °C).

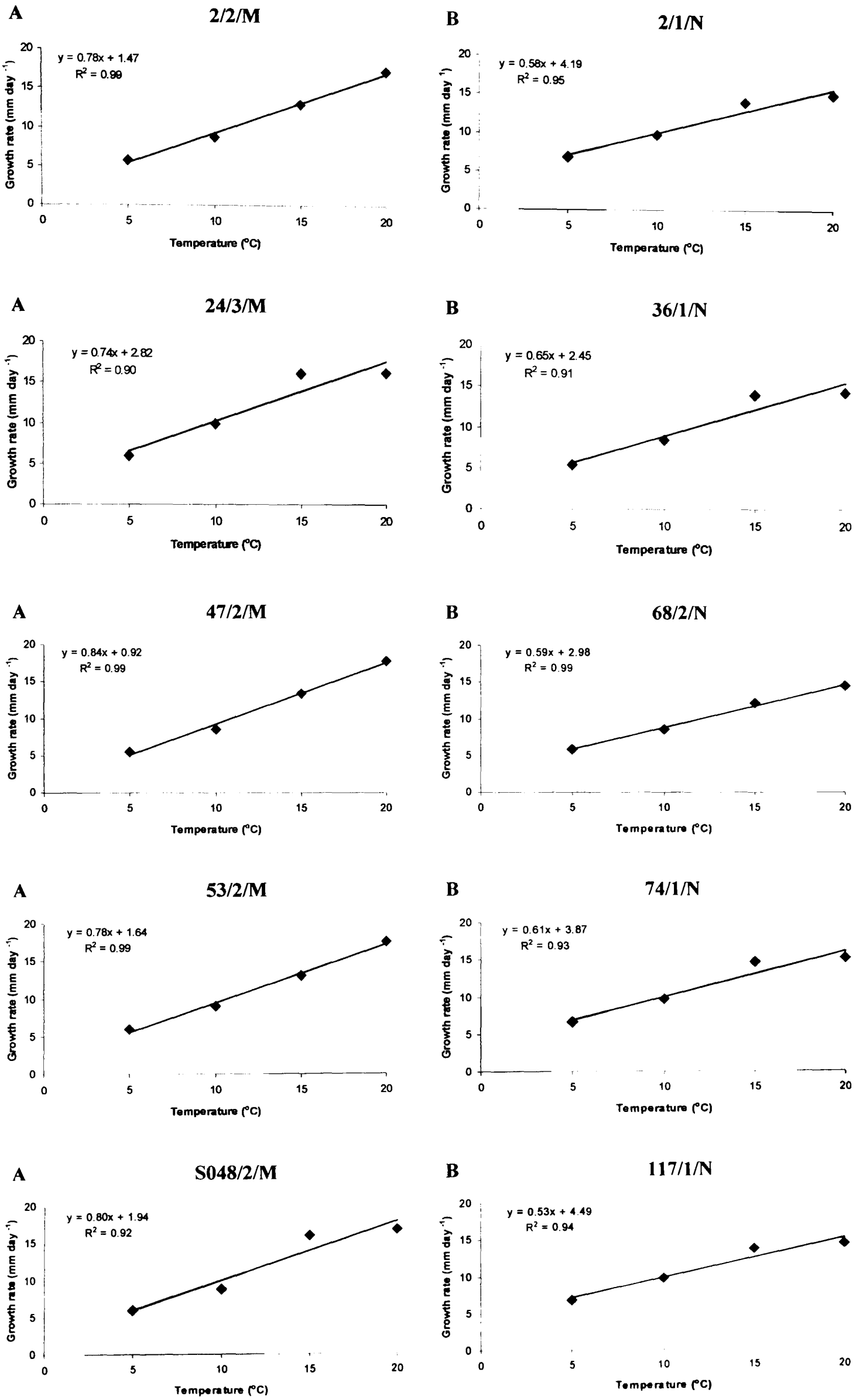


Figure 2. Effect of temperature on the growth of five *Microdochium nivale* var. *majus* and var. *nivale* isolates growth on 5 % w/w wheat flour agar over a period of 8 days incubation in darkness. **A** – var. ***majus*** isolates, **B** – var. *nivale* isolates.

Relationship between *in vitro* growth rate of *Microdochium nivale* sub-species and rate of imbibition, rate of emergence and seedling fresh weight increase of winter wheat

Microdochium nivale var. *majus* had a greater fungus:seedling growth rate ratio than var. *nivale* at GS 01 (Table 12) and GS 10 (Table 13) except at 5 °C. At GS 01, the fungus:seedling growth rate ratio was significantly ($P < 0.05$) in favour of *M. nivale* growth at -1.5 MPa (0.329) compared to 0 MPa (0.280). The fungus:seedling growth rate ratio was significantly ($P < 0.05$) in favour of *M. nivale* growth at 5 °C (0.151) but in favour of seedling growth at 10 °C (0.343) and 15 °C (0.4202). The *M. nivale* sub-species x temperature interaction was significant ($P = 0.018$). At 15 °C, var. *majus* had a significantly higher ($P < 0.05$) fungus:seedling growth rate ratio (0.438) than var. *nivale* (0.403). At 5 °C, var. *nivale* had a significantly higher ($P < 0.05$) fungus:seedling growth rate ratio (0.162) than var. *majus* (0.140).

Table 12. Effect of temperature and water potential (MPa) on the fungus:seedling growth rate ratio for *Microdochium nivale* sub-species and winter wheat (cv. Cadenza) at GS 01 in Petri dishes in controlled environments.

	fungus:seedling growth rate ratio					
	5 °C		**10 °C**		**15 °C**	
sub-spp.	**0 MPa**	**-1.5 MPa**	**0 MPa**	**-1.5 MPa**	**0 MPa**	**-1.5 MPa**
majus	0.131	0.150	0.326	0.365	0.393	0.483
nivale	0.151	0.173	0.321	0.360	0.362	0.444
majus[1]	0.361	0.388	0.571	0.604	0.625	0.694
nivale[1]	0.388	0.416	0.567	0.600	0.599	0.666
LSD ($P < 0.05$) = 0.0368, SED = 0.0181, DF = 34, %cv = 4.7						

1 data square root transformed.
Seedlot **1** (cv. Cadenza; 0 % infection) see Table 2; page 33.
Fungus:seedling growth rate ratio = $\frac{\textit{in vitro}\text{ growth rate}}{\text{increase in seedling fresh weight}}$

At GS 10, the fungus:seedling growth rate ratio was significantly ($P < 0.05$) in favour of *M. nivale* growth in dry soil (0.090) above wet soil (0.057). The fungus:seedling growth rate ratio was significantly ($P < 0.05$) in favour of *M. nivale* growth at 10 and 15 °C. At 15 °C, the fungus:seedling growth rate ratio significantly favoured ($P < 0.05$) seedling

growth in dry soil (0.069) but changed in favour of *M. nivale* growth in wet soil (0.158). The sub-species x temperature interaction was significant ($P = 0.037$). At 5 °C, var. *nivale* had a significantly higher ($P < 0.05$) fungus:seedling growth rate ratio (0.043) than var. *majus* (0.037). The opposite but not significant trend occurred at 15 °C (var. *majus* 0.118: var. *nivale* 0.109).

Table 13. Effect of temperature and soil water content on the fungus:seedling growth rate ratio for *Microdochium nivale* sub-species and winter wheat (cv. Cadenza) at GS 10 in tray trials in controlled environments.

	fungus:seedling growth rate ratio					
	5 °C		**10 °C**		**15 °C**	
sub-spp.	**wet**	**dry**	**wet**	**dry**	**wet**	**dry**
majus	0.035	0.039	0.064	0.070	0.072	0.165
nivale	0.040	0.045	0.063	0.069	0.066	0.151
majus[1]	-3.37	-3.25	-2.75	-2.68	-2.63	-1.81
nivale[1]	-3.22	-3.11	-2.77	-2.69	-2.72	-1.90

LSD ($P < 0.05$) = 0.177, SED = 0.087, DF = 34, %cv = 4.5

1 data natural logarithm transformed.
Wet soil – watered heavily; dry soil – 15 % w/w soil water content.
Seedlot **1** (cv. Cadenza; 0 % infection) see Table 2; page 33.
Fungus:seedling growth rate ratio = $\frac{\textit{in vitro}\text{ growth rate}}{\text{increase in seedling fresh weight}}$

Effect of delayed imbibition on amount of *Microdochium nivale* DNA in winter wheat seeds

Time in -4 MPa solution had no effect on seedling biomass increase. Seedling biomass for seedlot 2 (cv. Cadenza; 29 % infection) increased by 25 % compared with 31 % for seedlot 6 (cv. Equinox; 88 % infection). The quantity of *M. nivale* DNA in the cv. Equinox seedlot was lower than in the cv. Cadenza seedlot. The amount of *M. nivale* DNA did increase in imbibed seeds. The quantity of *M. nivale* DNA in seeds of seedlot 2 did not increase consistently with time in -4 MPa solution (Figure 3). In seeds of seedlot six, the amount of *M. nivale* DNA increased fairly linearly after 30, 40 and 50 days in -4 MPa solution (Figure 4).

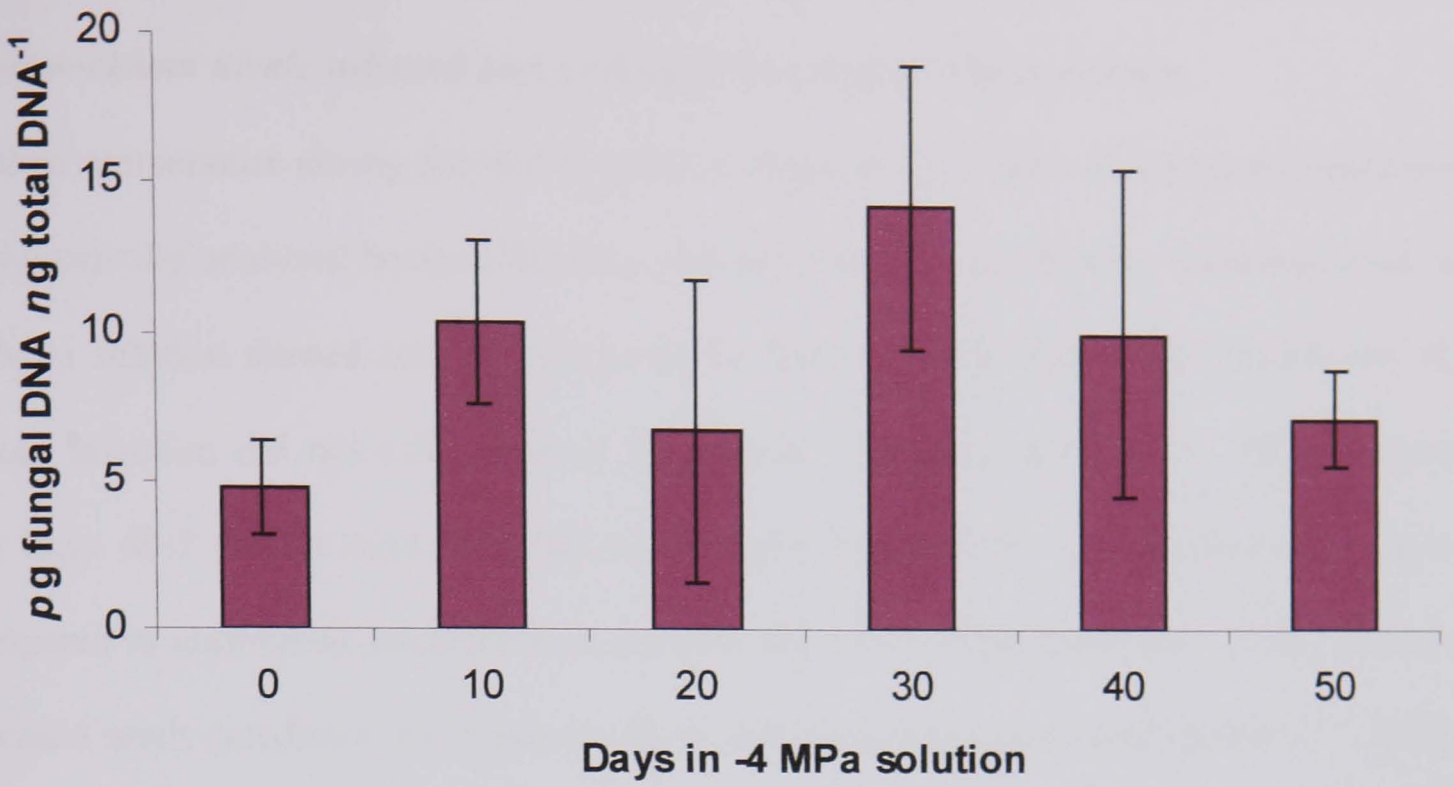


Figure 3. Effect of presoaking winter wheat seeds (cv. Cadenza) in -4 MPa solution at 5 °C on amount of seed-borne *Microdochium nivale* DNA.
Vertical lines represent SE.
Seedlot **2** (cv. Cadenza; 29 % infection) see Table 2; page 33.

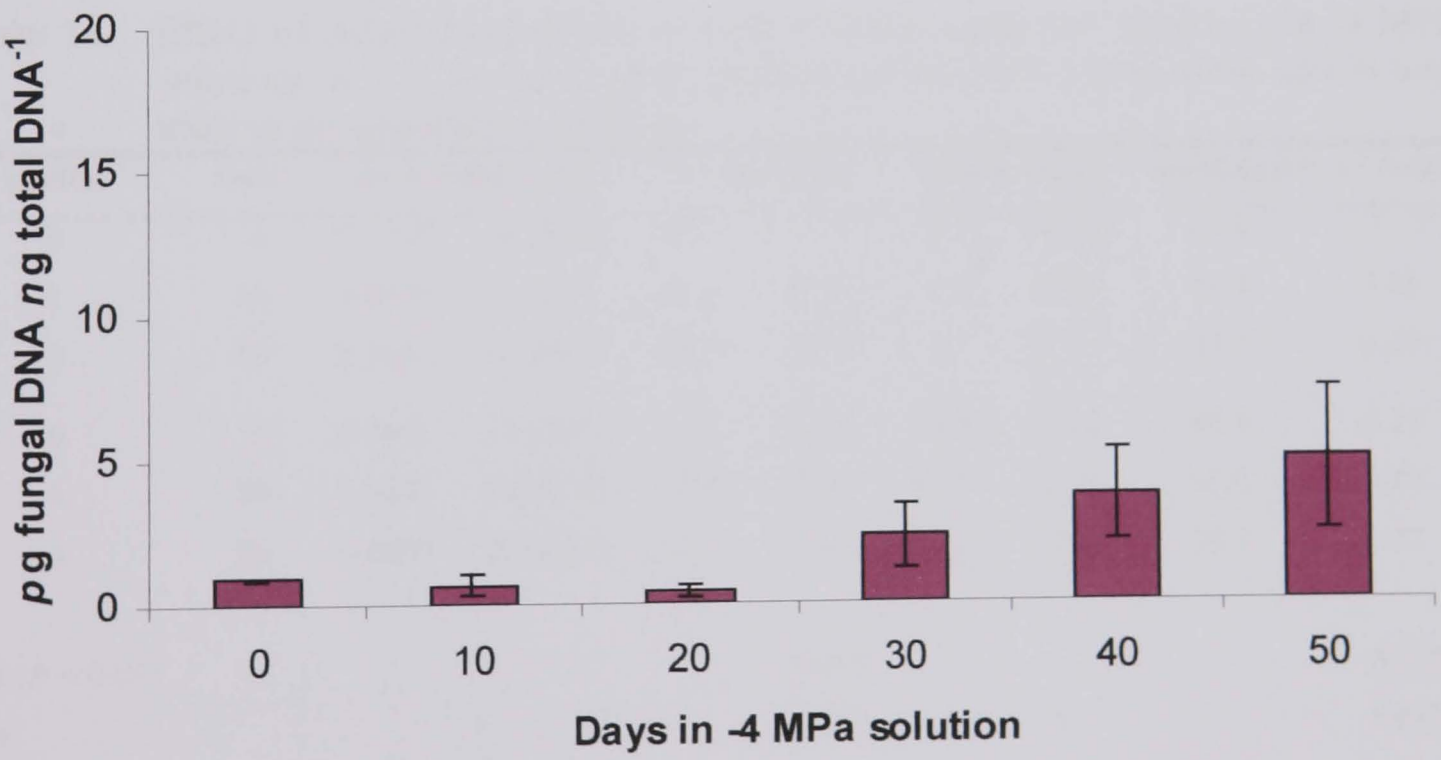


Figure 4. Effect of presoaking winter wheat seeds (cv. Equinox) in -4 MPa solution at 5 °C on amount of seed-borne *Microdochium nivale* DNA.
Vertical lines represent SE.
Seedlot **6** (cv. Equinox; 88 % infection) see Table 2; page 33.

Effect of delayed imbibition on seedling growth and seedling blight severity from *Microdochium nivale* infected and pathogen-free winter wheat seedlots

Median temperature during the trial is given in Appendix B. Rate of emergence could not be statistically analysed because the data was not normally distributed. Increased time in -4 MPa solution slowed rate of emergence for both seedlots (Table 14). Seed-borne *M. nivale* infection did not affect rate of emergence. Imbibing seeds in -4 MPa solution (30 days 69.7 %; 50 days 67.5 %) significantly reduced ($P < 0.05$) final emergence compared to unimbibed seeds for both seedlots (89.1 %). Final emergence (70.6 %) from diseased seeds (seedlot 6; cv. Equinox; 88 % infection) was significantly lower ($P < 0.05$) than pathogen-free seeds (seedlot 3; cv. Equinox; 0 % infection; 80.3 % emergence). Disease index data could not be statistically analysed because the data was not normally distributed. Diseased seeds (seedlot 6) gave rise to more heavily diseased seedlings than pathogen-free seeds (seedlot 3).

Table 14. Effect of delayed imbibition of winter wheat seeds (cv. Equinox) in -4 MPa solutions at 5 °C on subsequent seedling growth (GS 12) in moist soil in tray trials in an unheated polytunnel.

seedlot	time[1]	rate of emergence[2]		% emergence		disease index		seedling dry wt (mg)	
3	0	0.1108	(0.0004)	95.0	9.75[3]	5.2	(0.9)	43.8	-3.13[4]
3	30	0.0970	(0.0013)	69.5	8.34	5.9	(0.8)	46.0	-3.08
3	50	0.0863	(0.0008)	76.5	8.74	7.7	(1.4)	41.5	-3.19
6	0	0.1051	(0.0027)	83.2	9.12	55.8	(1.5)	40.5	-3.21
6	30	0.0973	(0.0032)	70.0	8.35	46.7	(2.7)	41.0	-3.20
6	50	0.0893	(0.0004)	58.5	7.62	44.1	(4.4)	36.3	-3.32
LSD ($P < 0.05$)				-	0.639			-	0.123
SED				-	0.304			-	0.059
DF				-	18			-	18
%cv				-	5.0			-	2.6

1 days in -4 MPa.
2 day^{-1}.
3 data square root transformed.
4 data natural logarithm transformed.
Numbers in parentheses represent SE.
Seedlot 3 (cv. Equinox; 0 % infection), seedlot 6 (cv. Equinox; 88 % infection) see Table 2; page 33.

Seedling blight disease index declined slightly with increased time in -4 MPa solution for diseased seeds. Seedling dry weight was significantly ($P < 0.05$) reduced by soaking seeds for 50 days (38.9 mg) in -4 MPa solution (unimbibed seeds 42.1 mg; seeds soaked for 30 days 43.5 mg). Seedlings from seedlot 3 (43.8 mg) were significantly ($P < 0.05$) heavier than seedlings from seedlot 6 (39.3 mg).

Effect of temperature and soil water content on amount of *Microdochium nivale* DNA and seedling growth from untreated diseased winter wheat seedlots

Temperature significantly ($P < 0.001$) affected rate of imbibition for seedlot 2 (cv. Cadenza; 29 % infection). Rate of imbibition was fastest at 15 °C (0.437 day^{-1}), significantly slower ($P < 0.05$) at 10 °C (0.319 day^{-1}) and slowest at 5 °C (0.209 day^{-1}). Rate of imbibition was significantly faster ($P < 0.05$) at 0 MPa (0.406 day^{-1}) than -1.5 MPa (0.236 day^{-1}). Rate of imbibition was significantly faster ($P < 0.05$) at 0 MPa than -1.5 MPa across all temperatures (Table 15). Temperature did not significantly affect final imbibition ($P = 0.168$). Final imbibition was significantly greater ($P < 0.05$) at 0 MPa (95.3 %) than -1.5 MPa (91.8 %). The temperature x water potential interaction was significant ($P = 0.038$). At 15 °C, final imbibition was significantly higher ($P < 0.05$) at 0 MPa but the opposite trend occurred at 5 °C (Table 15).

Temperature significantly ($P < 0.001$) affected rate of imbibition for seedlot 6 (cv. Equinox; 88 % infection). Rate of imbibition was fastest at 15 °C (0.547 day^{-1}), significantly slower ($P < 0.05$) at 10 °C (0.326 day^{-1}) and slowest at 5 °C (0.185 day^{-1}). Rate of imbibition was significantly faster ($P < 0.05$) at 0 MPa (0.455 day^{-1}) than -1.5 MPa (0.250 day^{-1}) across all temperatures (Table 16). Final imbibition was not significantly affected by temperature ($P = 0.284$), water potential ($P = 0.344$) or the temperature x water potential interaction ($P = 0.256$).

Table 15. Effect of temperature and water potential (MPa) on rate of imbibition (day^{-1}) and final imbibition (GS 01) of a winter wheat seedlot (cv. Cadenza) with *Microdochium nivale* infection in Petri dishes in controlled environments.

temperature	water potential	rate of imbibition		% imbibition	
5 °C	0	0.254	-1.372[1]	96.5	79.8[2]
	-1.5	0.162	-1.822	92.8	74.5
10 °C	0	0.396	-0.925	93.8	75.6
	-1.5	0.241	-1.142	95.0	77.8
15 °C	0	0.568	-0.567	95.5	78.2
	-1.5	0.306	-1.188	87.8	69.6
LSD ($P < 0.05$)		-	0.0727	-	5.18
SED		-	0.0346	-	2.47
DF		-	18	-	18
%cv		-	4.0	-	4.6

1 data angular transformed.
2 data natural logarithm transformed.
Seedlot **2** (cv. Cadenza; 29 % infection) see Table 2; page 33.

Temperature significantly ($P < 0.001$) affected rate of emergence for seedlot 2 (cv. Cadenza; 29 % infection). Rate of emergence was quickest at 15 °C (0.090 day^{-1}), significantly slower ($P < 0.05$) at 10 °C (0.067 day^{-1}) and slowest at 5 °C (0.028 day^{-1}). Rate of emergence was significantly faster ($P < 0.05$) at 0 MPa (0.076 day^{-1}) than -1.5 MPa (0.048 day^{-1}). Rate of emergence was significantly faster ($P < 0.05$) at 0 MPa than -1.5 MPa at 15 °C and 5 °C. Temperature significantly affected final emergence ($P = 0.018$). Final emergence at 15 °C (80 %) and 5 °C (77 %) was significantly above ($P < 0.05$) emergence at 10 °C (69 %). The temperature x soil water interaction was

Table 16. Effect of temperature and water potential (MPa) on rate of imbibition (day^{-1}) and final imbibition (GS 01) of a winter wheat seedlot (cv. Equinox) with *Microdochium nivale* infection in Petri dishes in controlled environments.

temperature	water potential	rate of imbibition		% imbibition	
5 °C	0	0.227	-1.49[1]	87.3	69.4[2]
	-1.5	0.142	-1.95	86.8	68.7
10 °C	0	0.410	-0.89	83.5	66.1
	-1.5	0.242	-1.42	85.0	67.2
15 °C	0	0.227	-1.49	87.3	69.4
	-1.5	0.142	-1.95	86.8	68.7
LSD ($P < 0.05$)		-	0.121	-	4.95
SED		-	0.058	-	2.36
DF		-	18	-	18
%cv		-	6.9	-	4.9

1 data natural logarithm transformed.
2 data angular transformed.
Seedlot **6** (cv. Equinox; 88 % infection) see Table 2; page 33.

significant ($P = 0.005$). At 15 °C. final emergence from wet soil was significantly above ($P < 0.05$) dry soil. the opposite trend occurred at 10 and 5 °C (Table 17).

Table 17. Effect of temperature and soil water content on rate of emergence (day^{-1}) and final emergence (GS 10) from a winter wheat seedlot (cv. Cadenza) with *Microdochium nivale* infection in tray trials in controlled environments.

temperature	soil water	rate of emergence		% emergence	
5 °C	wet	0.032	-3.46[1]	72	4.27[1]
	dry	0.024	-3.73	82	4.40
10 °C	wet	0.062	-2.79	62	4.12
	dry	0.072	-2.64	76	4.32
15 °C	wet	0.133	-2.02	86	4.46
	dry	0.047	-3.05	74	4.30
LSD ($P < 0.05$)		-	0.211	-	0.150
SED		-	0.101	-	0.071
DF		-	18	-	18
%cv		-	4.8	-	2.3

1 data natural logarithm transformed.
Wet soil – heavily watered; dry soil – 15 % w/w soil water content.
Seedlot **2** (cv. Cadenza; 29 % infection) see Table 2; page 33.

Rate of emergence for seedlot 6 (cv. Equinox; 88 % infection) could not be analysed because the data was not normally distributed. Temperature significantly affected final emergence ($P = 0.002$). Final emergence at 15 °C (61 %) and 10 °C (59 %) was significantly above ($P < 0.05$) final emergence at 5 °C (44 %). The temperature x soil water interaction was significant ($P = 0.004$). Final emergence from wet soil was only significantly above ($P < 0.05$) final emergence from dry soil at 15 °C (Table 18).

There were no correlations between the amount of fungal DNA at GS 01 or GS 10 and seedling growth for either seedlot. Differences between the seedlots in the fungal DNA:seedling biomass ratio were not consistent at GS 01 or GS 10. However, ratios were similar within the seedlots. For seedlot 2, no conditions were favourable for *M. nivale* growth at GS 01 (Figure 5a) and only dry soil provided favourable conditions for *M. nivale* growth at GS 10 (Figure 6a). For seedlot 6, dry soil appeared to provide more favourable conditions for *M. nivale* growth at GS 01 (Figure 5b) and GS 10, measured by competitive PCR (Figure 6b).

Table 18. Effect of temperature and soil water content on rate of emergence (day^{-1}) and final emergence (GS 10) from a winter wheat seedlot (cv. Equinox) with *Microdochium nivale* infection in tray trials in controlled environments.

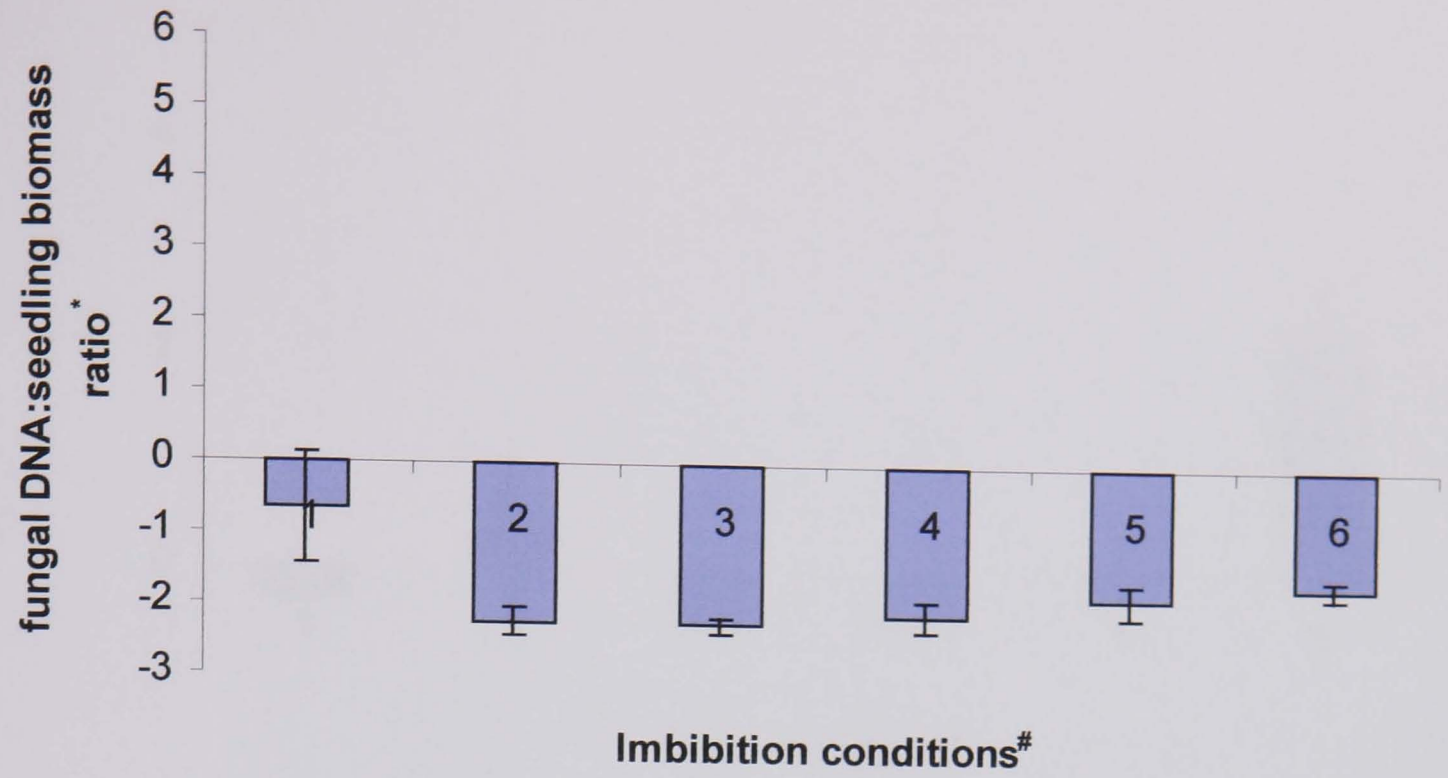
temperature	soil water	rate of emergence		% emergence	
5 °C	wet	0.029	(0.001)	48	44.0[1]
	dry	0.024	(0.001)	38	39.0
10 °C	wet	0.064	(0.008)	59	50.0
	dry	0.083	(0.005)	59	50.3
15 °C	wet	0.153	(0.012)	77	61.2
	dry	0.051	(0.003)	45	42.2
LSD ($P < 0.05$)				-	7.68
SED				-	3.66
DF				-	18
%cv				-	10.8

1 data angular transformed.
Wet soil – heavily watered; dry soil – 15 % w/w soil water content.
Numbers in parentheses represent SE.
Seedlot **6** (cv. Equinox; 88 % infection) see Table 2; page 33.

DISCUSSION

Parry *et al.* (1995) and Turner *et al.* (1999) have observed an increase in *M. nivale* var. *majus* incidence on winter wheat in UK field studies. Perry (1986) described three *M. nivale* isolates causing different disease severity and shoot length reductions of barley seedlings (cv. Golden Promise) from artificial soil-borne inoculum. However, there were insufficient isolates to determine specific trends and the sub-species classification were unknown. Hare (1997) tested the pathogenicity of four var. *majus* and four var. *nivale* isolates to winter wheat seedlings (cv. Brigadier) at 6 °C. *Microdochium nivale* var. *majus* isolates from surface-inoculated seeds, significantly reduced final emergence, coleoptile, shoot and root lengths and increased the disease index more than var. *nivale* inoculated seeds. *Microdochium nivale* var. *nivale* isolates only significantly reduced root lengths below untreated seeds. There is additional evidence for var. *majus* having relatively greater pathogenicity to wheat plants. For example, three *M. nivale* var. *majus* isolates produced significantly larger lesions on detached wheat leaves in a shorter time than three isolates of var. *nivale* (Diamond & Cook, 1997). In a controlled environment trial, increased quantities of *M. nivale* var. *majus* DNA were detected in wheat stem-bases after inoculation at GS 12 with mixed sub-species mycelium at 15 °C (Simpson *et al.*, 2000). In

A



B

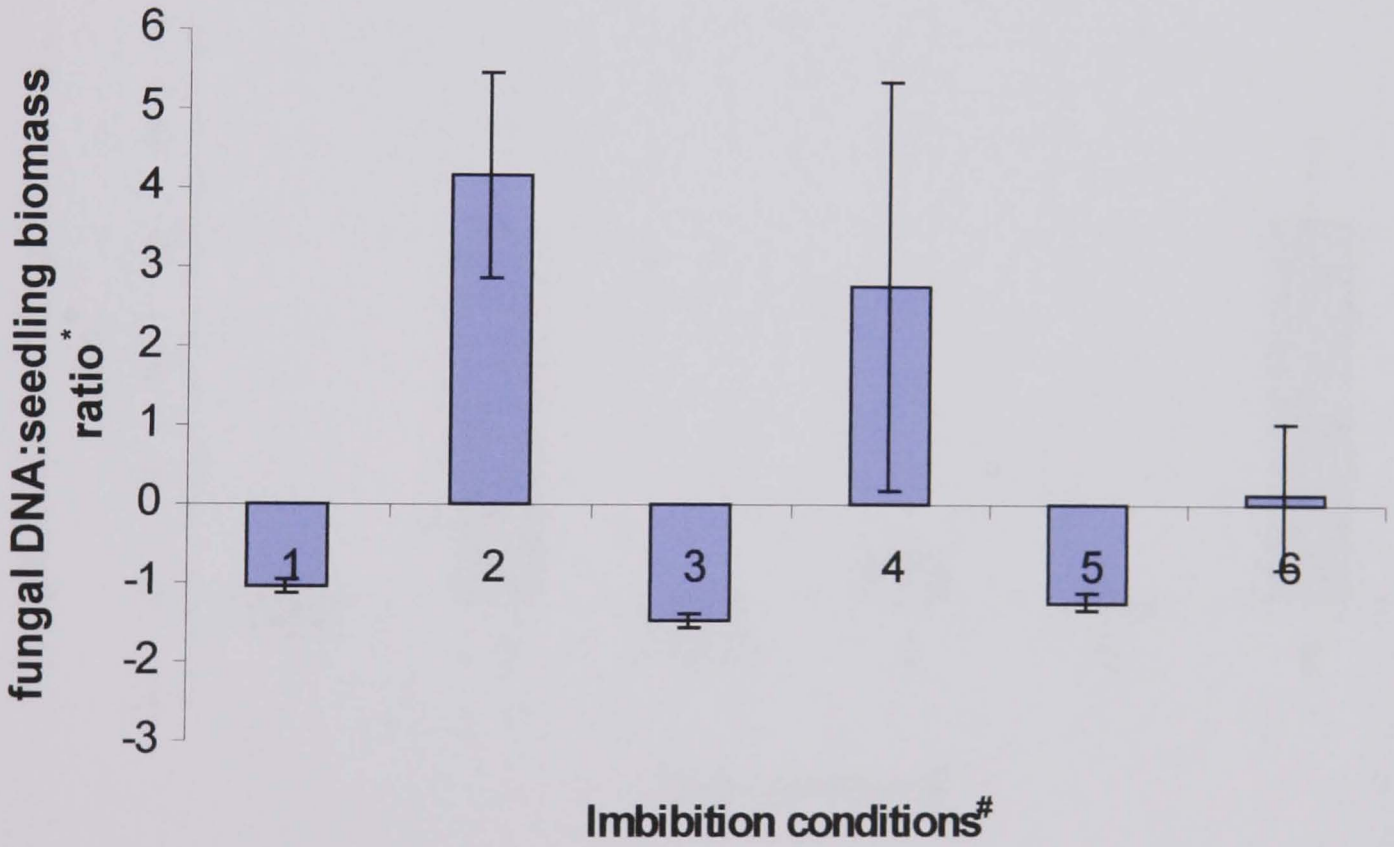


Figure 5. Effect of temperature and water potential (MPa) on the *Microdochium nivale* DNA:seedling biomass ratio for winter wheat seedlots (cv. Cadenza and cv. Equinox) at GS 01.

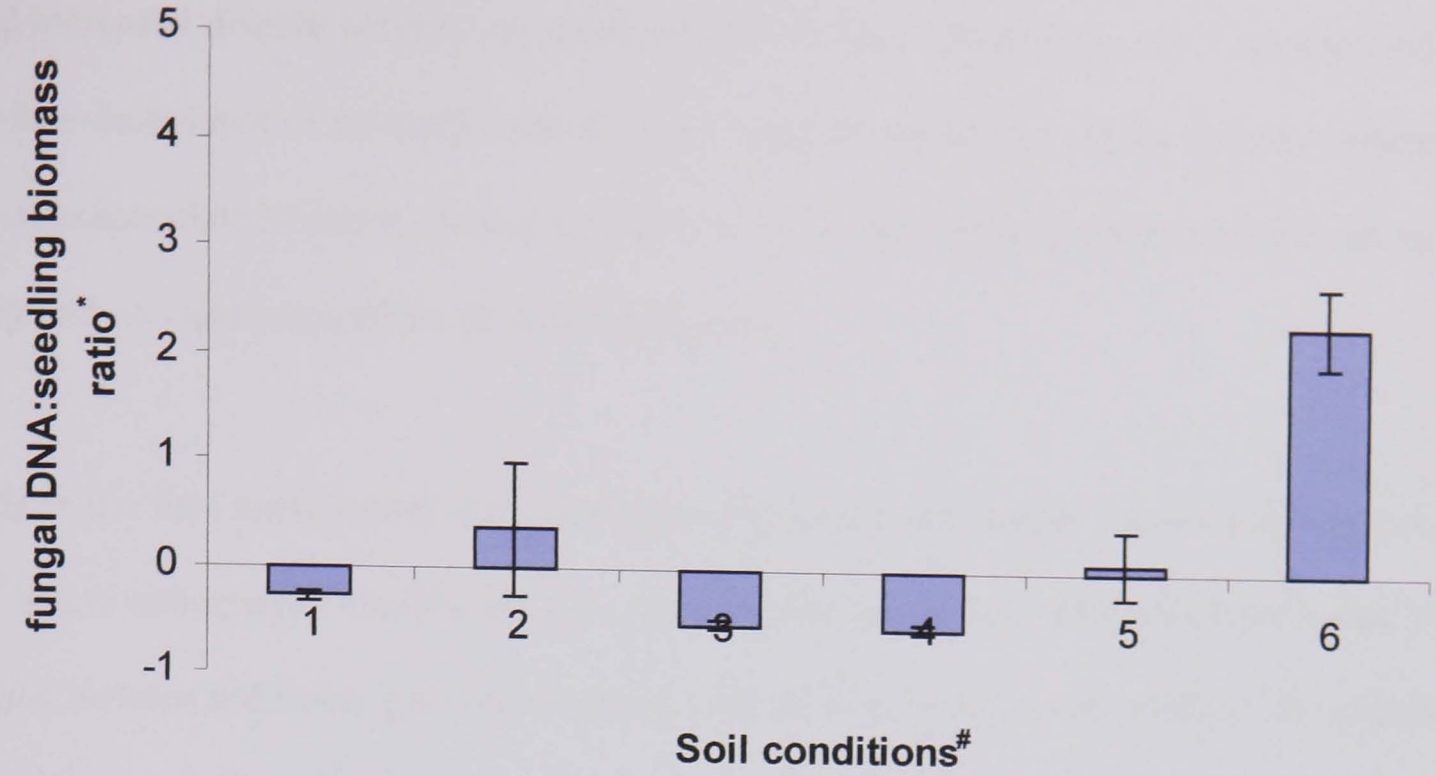
1 = 15 °C:0 MPa, 2 = 15 °C:-1.5 MPa, 3 = 10 °C:0 MPa, 4 = 10 °C:-1.5 MPa, 5 = 5 °C:0 MPa, 6 = 5 °C:-1.5 MPa.

* calculated by $\frac{\text{\% increase in fungal DNA}}{\text{\% increase in seedling fresh wt increase}}$

Vertical lines represent SE.

A – seedlot **2** (cv. Cadenza; 29 % infection) **B** – seedlot **6** (cv. Equinox; 88 % infection) see Table 2; page 33.

A



B

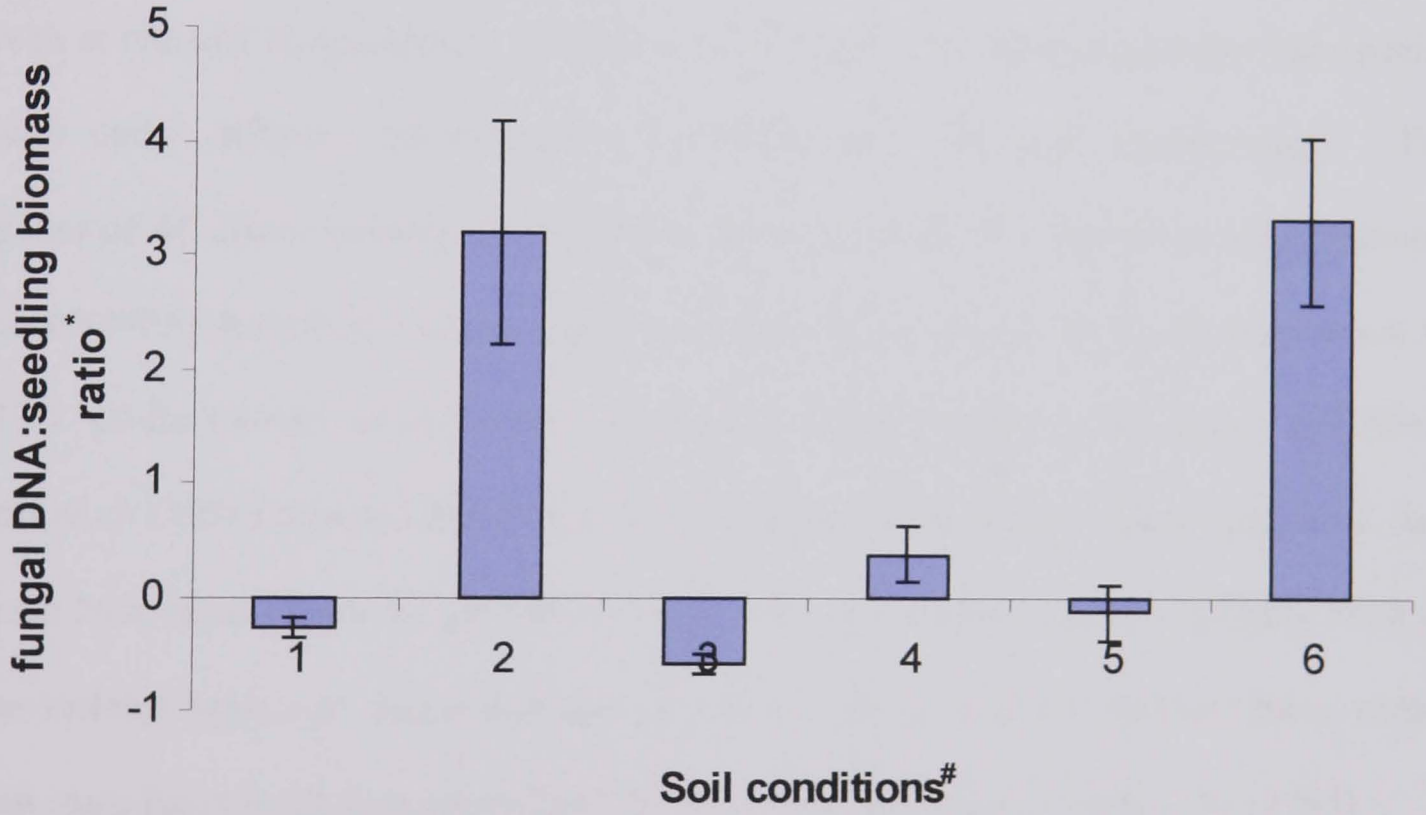


Figure 6. Effect of temperature and soil water content (% w/w) on the *Microdochium nivale* DNA:seedling biomass ratio for winter wheat seedlots (cv. Cadenza and cv. Equinox) at GS 10.

1 = 15 °C:wet soil, 2 = 15 °C:15 % w/w soil water content, 3 = 10 °C:wet soil, 4 = 10 °C:15 % w/w soil water content, 5 = 5 °C:wet soil, 6 = 5 °C: 15 % w/w soil water content.

* calculated by $\frac{\text{\% increase in fungal DNA}}{\text{\% increase in seedling fresh wt increase}}$

Vertical lines represent SE.

A – seedlot **2** (cv. Cadenza; 29 % infection) **B** – seedlot **6** (cv. Equinox; 88 % infection) see Table 2; page 33.

a controlled environment investigation, Glynn (2002) observed reduced final emergence and increased disease severity on seedlings from winter wheat seeds (cv. Cadenza), either surface-inoculated or naturally infected with eight *M. nivale* var. *majus* isolates compared to an uninoculated control. However, eight *M. nivale* var. *nivale* isolates tested in the same way had no significant effect on final emergence.

This is the first documented investigation into possible reasons for reported differences in *M. nivale* sub-species distribution and pathogenicity in wheat. *Microdochium nivale* var. *majus* isolates had faster growth between 5 and 20 °C than var. *nivale* isolates. In addition, the fungus:seedling growth rate ratio favoured var. *majus* above var. *nivale* at 10 and 15 °C. These results may suggest that increased var. *majus* incidence above var. *nivale* on winter wheat may be due to its faster growth in the spring because var. *nivale* has faster growth at reduced temperatures. Further work is required to what importance sub-species growth under different environmental conditions plays in their epidemiology. The response of *M. nivale* isolates to temperature is in agreement with previous investigations. Bennett (1933) described a linear relationship between *in vitro* growth and temperature (0 to 32.5 °C) for two *M. nivale* isolates. However, the methodology was poorly described. Pettitt *et al.* (1996) reported that five *M. nivale* isolates from winter wheat stem-bases, had a mean base temperature for growth of 1.5 °C on straw extract agar. It is likely most of these isolates were var. *majus* because of 144 *M. nivale* isolates obtained from winter wheat stem-bases at 30 sites across the UK, 70 % were var. *majus* (Parry *et al.*, 1995).

This investigation confirmed that overall rate of imbibition, final imbibition, rate of emergence and final emergence are greatest in warm wet environments and are reduced in cold dry environments for *M. nivale* diseased and pathogen-free winter wheat seeds. Seed-borne *M. nivale* is more pathogenic to winter wheat seedlings at low temperatures

(<10 °C), measured by reduced final emergence (Hare *et al.*, 1995) whilst *F. graminearum* and *F. culmorum* are more pathogenic to seedlings at high temperatures. For example, Gilbert *et al.* (1997) recorded increased germination of *F. graminearum* infected spring wheat seedlots at 5 °C rather than 20 / 15 °C. In this investigation, fungus:seedling growth rate ratios were more in favour of the fungus at 10 and 15 °C. However, highest emergence from diseased seeds occurred at 15 °C. There was also no correlation between the amount of fungal DNA and final imbibition or emergence across a range of temperatures and soil water regimes. These results do not corroborate the hypothesis of Cristani (1992) who from trials with naturally infected *M. nivale* seedlots, proposed fungus growth was greatly hindered by high temperatures which also increased early seedling growth. However, the *in vitro* growth trials in this investigation suggest that this is unlikely to occur. It is more likely that high temperatures favour seedling growth over *M. nivale* infection, whilst low temperatures favour *M. nivale* infection over seedling growth in a more complex manner than can be explained by pathogen and host growth rates. Indeed the influence of water potential on imbibition and soil water content on emergence suggest this is indeed the case. Hare & Parry (1996) demonstrated a good relationship between mean Ψ_m (-0.175 to -0.135 MPa) and rate of seedling emergence, final emergence and post-emergent symptoms for winter wheat seeds (cv. Mercia) surface-inoculated with *F. culmorum* at 20 °C. There was also an inverse relationship between mean Ψ_m and pre-emergent death, implying a mechanism of disease escape through increased seedling growth in wetter soil. However, there was no consideration of how *F. culmorum* growth was affected under these conditions. Water potential and soil water content influenced the fungus biomass:seedling growth rate ratio less than temperature although low water potential and dry soil slowed rate of imbibition and emergence respectively. This may be an avenue for further research.

Similar investigations have demonstrated that relative growth rates can partially predict conditions under which most severe disease arises. Maximal growth of *F. graminearum in vitro* (24 to 28 °C) has been shown to correlate to maximum disease incidence on wheat seedlings grown from surface-inoculated seeds between 8 and 36 °C (Dickinson, 1923). At these temperatures, blight incidence was increased at all temperatures by low soil water contents (30, 45 and 60 % MWHC). In the experiments of Leach (1947), final seedling emergence from infested soils at different temperatures correlated closely with the ratio between coefficient of emergence and *in vitro* fungus growth rate for several damping-off host-pathogen interactions. Increased germination and subsequent growth of winter wheat (cv. Viking) through soaking seeds for 1 to 12 h prior to inoculation and planting appeared to reduce the opportunity for *F. culmorum* infection from surface-inoculated seeds. Seedlings from seeds with reduced vigour had increased disease severity (Malalasekera & Colhoun, 1968). From the very limited data presented, the host-pathogen growth ratio (uninoculated plant fresh weight to radial growth rate) most favoured the host after three hours soaking.

This investigation has demonstrated that delayed seedling imbibition (30 days for cv. Cadenza; 40 and 50 days for cv. Equinox), increases the amount of *M. nivale* DNA in seeds, although for both seedlots responses to time in solution were inconsistent. Whilst the duration of seed imbibition was far above that likely to occur under natural conditions, this does provide evidence that slower seedling growth allows the amount of seed-borne *M. nivale* DNA to increase. Reductions in *M. nivale* DNA in seedlot 2 (cv. Cadenza; 29 % infection) after 40 and 50 days soaking in -4 MPa solution, may be due to fungus lysis. Delayed imbibition adversely affected seedling growth from pathogen-free seeds and there was no effect of seed-borne *M. nivale* infection on seedling growth and disease development. Different trends between seedlots may be due to cultivar differences in

susceptibility to *Fusarium* seedling blight (Arseniuk *et al.*, 1993; Pavlova & Srobarova, 1997), site of infection which is at present unknown or the quantity of infection within the seed.

CHAPTER 6

Effects of timing, duration and severity of freezing on seedling emergence and vigour and seedling blight severity from seedlots with *Microdochium nivale* infection

INTRODUCTION

Under UK conditions, winter wheat seedlings are likely to be exposed to soil temperatures below 0 °C. *Microdochium nivale* seedling blight from seed-borne (Hare, 1997) and soil-borne (Perry, 1986) inoculum is more severe at low temperatures. Insufficient research has been conducted into the effect of freezing on seedlings exposed to *M. nivale*. Only Bateman (1976) has reported that maintaining newly emerged wheat and barley seedlings from infected seeds at 0 to 1 °C for several weeks, increased *M. nivale* isolation from coleoptiles.

Despite the lack of evidence for the effect of freezing on wheat seedlings from naturally infected *M. nivale* seeds, freezing does increase seedling blight incidence and severity on oats and barley from surface-inoculated seeds and soil-borne inoculum. Freezing for 4 h at -6 °C air temperatures on four occasions at weekly intervals beginning one month after planting, increased *M. nivale* isolation incidence from seedling mesocotyls and roots of untreated oat seeds (Rawlinson & Colhoun, 1969). Freezing for 48 h at -2 °C; 10 days after planting of barley seeds surface-inoculated with *M. nivale* conidia, increased coleoptile lesion index and reduced seedling height compared to seedlings exposed to 2 °C or maintained at 10 °C (Perry, 1986). In a further experiment, freezing (-2 °C) 4-7 days after sowing caused an increase in the incidence of coleoptile infection and severest lesion index (Perry, 1986). However, soil water contents were not presented. Seedlings from uninoculated seeds exposed to -2 °C also had coleoptile lesions suggesting these may be freezing injuries or infection from soil-borne *M. nivale*.

In summary, the limited evidence available suggests that freezing during emergence, delays seedling growth increasing the opportunity for infection. However, there is a paucity of information regarding the effects of freezing on seedling blight severity of

wheat from seed-borne *M. nivale* infection. The aims of this chapter were to investigate (i) the effect of timing, duration and severity of freezing on seedling blight severity and seedling vigour from seed-borne *M. nivale* infection. (ii) whether *M. nivale* infection pre-disposes seedlings to freezing injury by lowering the sub-lethal threshold or whether freezing pre-disposes the seedling to *M. nivale* infection. (iii) if carboxin + thiram control of seedling blight caused by seed-borne *M. nivale* is affected by freezing timing, duration and severity. (iv) the effect of soil water content on the above interactions in 16 controlled environment investigations.

MATERIALS & METHODS

Experiments were designed to test the following hypotheses: (i) timing, duration and severity of freezing has no effect on seedling blight severity, (ii) reduced soil water content does not increase the effect of freezing on seedling blight and (iii) carboxin + thiram seed treatment has no effect on seedling blight severity from seed-borne infection under controlled environmental conditions. Seedlots 2 (cv. Cadenza; 29 % infection), 6 (cv. Equinox; 88 % infection) and 10 (cv. Riband; 73 % infection) (Table 2; page 33) were used in this investigation to provide a range of *M. nivale* seed-borne infections. For a summary of each experiment refer to Table 19.. Ten surface-sterilised untreated or carboxin + thiram treated seeds of each seedlot were planted 20 mm deep in glass jars (Table 3) containing 80 g of autoclaved John Innes No. 2 compost at 20 or 40 % w/w soil water content. The bottles were closed and incubated under 12 h light (9 μmol m^{-2} s^{-1} mean photon flux density) (11 °C) and 12 h darkness (7 °C). Freezing temperatures were applied 7, 14, 21 or 28 days after planting for 12 or 24 h at 0 or -5 °C (Table 19). After freezing, pots were returned to the incubator.

Table 19. Seedlots used for each freezing investigation.

experiment	seedlot	freezing severity	freezing duration (h)
1	2	-5 °C	12
2	2	-5 °C	24
3	6	-5 °C	12
4	6	-5 °C	24
5	6	0 °C	12
6	6	0 °C	24
7	10	0 °C	12
8	10	0 °C	24

Final emergence and disease severity (Table 4) were measured at GS 12 and seedling dry weight determined (Chapter 3). Rate of emergence (Equation 2) was calculated from daily plant counts. Five replicates were used for each treatment. Each experiment was repeated twice. Factorial analysis was conducted with rate of emergence, final emergence, disease index and seedling dry weight as variables and seed treatment, soil water regime and timing of freezing as factors. Where appropriate, data were transformed prior to analysis to ensure they were normally distributed. Regression analysis was conducted between rate of emergence, final emergence and disease severity.

RESULTS

Effect of freezing for 12 h at 0 °C and carboxin + thiram seed treatment on seedling growth and seedling blight severity from seed-borne *Microdochium nivale* infection

Seedlots 6 (cv. Equinox; 88 % infection) and 10 (cv. Riband; 73 % infection) exhibited different responses to freezing at 0 °C for 12 h. Freezing seven and 14 days after planting significantly ($P < 0.05$) slowed rate of emergence for seedlot 6 (Table 20). Emergence was significantly faster ($P < 0.05$) from wet soil (0.057 day^{-1}) than dry soil (0.046 day^{-1}). The seed treatment x soil water content interaction was significant ($P < 0.001$). Emergence was

significantly faster ($P < 0.05$) from carboxin + thiram treated seeds (0.053 day^{-1}) above untreated seeds (0.040 day^{-1}) only in dry soil.

Table 20. Effect of timing of 12 h freezing (0 °C) on rate of emergence (day^{-1}), final emergence, disease index and dry weight (mg) of winter wheat seedlings from a seedlot (cv. Equinox) with 88 % *Microdochium nivale* infection.

freezing timing*	soil water content (% w/w)	rate of emergence	% emergence		disease index		seedling dry wt
untreated seeds							
none	20	0.044	36	37**	42	40**	32
none	40	0.059	63	53	48	43	40
7	20	0.039	41	39	36	36	34
7	40	0.052	59	51	51	46	33
14	20	0.037	51	45	24	27	32
14	40	0.056	71	59	52	46	33
21	20	0.039	43	41	43	41	35
21	40	0.057	72	60	40	39	34
28	20	0.040	44	41	38	36	35
28	40	0.059	63	53	55	48	36
carboxin + thiram treated seeds							
none	20	0.055	85	69	18	23	34
none	40	0.062	80	65	18	24	36
7	20	0.054	83	69	23	28	32
7	40	0.054	81	66	17	24	33
14	20	0.049	77	66	25	29	32
14	40	0.059	81	67	22	27	33
21	20	0.052	72	60	18	23	35
21	40	0.057	80	66	13	19	32
28	20	0.054	86	70	10	15	34
28	40	0.059	89	75	11	17	33
LSD ($P < 0.05$)		0.0073	-	10.9	-	9.8	6.3
SED		0.0037	-	5.5	-	5.0	3.2
DF		179	-	179	-	179	179
%cv		15.9	-	21.4	-	35.2	21.2

* days after planting.
** data angular transformed.
Seedlot **6** (cv. Equinox; 88 % infection) see Table 2; page 33.

Timing of freezing did not significantly reduce final emergence ($P = 0.537$). Final emergence from carboxin + thiram treated seeds (81 %) was significantly above ($P < 0.05$)

untreated seeds (54 %). Final emergence from wet soil (74 %) was significantly above ($P < 0.05$) dry soil (62 %). Final emergence from carboxin + thiram treated seeds was significantly above untreated seeds across all freezing timings in both wet and dry soils ($P < 0.05$). There was no correlation between rate of emergence and final emergence.

Timing of freezing did not significantly increase the seedling disease index ($P = 0.794$). Carboxin + thiram treatment (18 %) significantly ($P < 0.05$) reduced disease below untreated seeds (43 %). Seedlings from untreated seeds (33 %) grown in wet soil had significantly more ($P < 0.05$) disease than seedlings grown in dry soil (28 %). Carboxin + thiram seed treatment significantly reduced ($P < 0.05$) the seedling disease index below untreated seeds in wet (carboxin + thiram treated seeds 17 %; untreated seeds 49 %) and dry soil (carboxin + thiram treated seeds 19 %; untreated seeds 37 %) across all freezing times. No correlations occurred between rate of emergence and disease severity. No consistent effects were observed for timing of freezing, seed treatment or soil water content on seedling dry weight.

For seedlot 10 (cv. Riband; 73 % infection) 12 h freezing at 0 °C seven days after planting significantly ($P < 0.05$) reduced rate of emergence (Table 21). Rate of emergence from carboxin + thiram treated seeds (0.055 day^{-1}) was significantly slower ($P < 0.05$) than from untreated seeds (0.059 day^{-1}). The timing of freezing x soil water content x seed treatment interaction was significant ($P = 0.045$). Freezing seven and 14 days after planting significantly reduced final emergence ($P < 0.05$). Final emergence was significantly increased ($P < 0.05$) from dry soil (86 %) compared to wet soil (78 %). There was no correlation between rate of emergence and final emergence. Disease index could not be statistically analysed due to a lack of normality. Freezing increased disease severity on seedlings grown in dry soil for untreated seeds. The opposite effect occurred for seedlings grown in wet soil. Carboxin + thiram treatment significantly reduced the disease index

compared to untreated seeds especially in wet soil. Soil water content had an inconsistent effect on untreated seeds. No correlations occurred between rate of emergence and disease severity.

Table 21. Effect of timing of 12 h freezing (0 °C) on rate of emergence (day^{-1}), final emergence, disease index and dry weight (mg) of winter wheat seedlings from a seedlot (cv. Riband) with 73 % *Microdochium nivale* infection.

freezing timing*	soil water content (% w/w)	rate of emergence	% emergence	disease index		seedling dry wt
untreated seeds						
none	20	0.0605	91	11.4	(4.5)	26.7
none	40	0.0617	86	27.4	(6.6)	27.4
7	20	0.0584	89	17.4	(3.6)	28.8
7	40	0.0542	80	17.9	(6.7)	26.7
14	20	0.0609	86	12.7	(4.6)	27.4
14	40	0.0572	72	11.1	(2.4)	31.4
21	20	0.0604	89	14.2	(3.4)	26.1
21	40	0.0590	84	21.5	(7.0)	26.7
28	20	0.0551	81	18.7	(6.2)	27.7
28	40	0.0602	75	8.8	(2.7)	30.0
carboxin + thiram treated seeds						
none	20	0.0559	90	3.9	(2.1)	29.3
none	40	0.0529	81	0.8	(0.3)	27.2
7	20	0.0544	87	9.3	(4.0)	26.8
7	40	0.0542	68	0.5	(0.3)	35.3
14	20	0.0534	83	1.9	(1.3)	28.9
14	40	0.0566	73	2.0	(1.0)	27.4
21	20	0.0574	84	4.1	(3.7)	29.7
21	40	0.0560	77	1.2	(0.8)	27.3
28	20	0.0536	82	3.4	(2.4)	28.2
28	40	0.0528	80	0.8	(0.6)	28.5
LSD ($P < 0.05$)		0.00475	11.9	-		4.37
SED		0.00241	6.0	-		2.21
DF		179	179	-		179
%cv		9.5	16.5			17.4

* days after planting.
Numbers in parentheses represent SE.
Seedlot **10** (cv. Riband; 73 % infection) see Table2; page 33.

Effect of freezing for 24 h at 0 °C and carboxin + thiram seed treatment on seedling growth and seedling blight severity from seed-borne *Microdochium nivale* infection

Seedlots 6 (cv. Equinox; 88 % infection) and 10 (cv. Riband; 73 % infection) exhibited different responses to freezing at 0 °C for 24 h. Freezing at 0 °C for 24 h seven days after planting significantly reduced ($P < 0.05$) rate of emergence from seedlot 6 (Table 22). Rate of emergence from wet soil (0.0535 day^{-1}) was significantly faster ($P < 0.05$) than from dry soil (0.0463 day^{-1}). Rate of emergence from untreated seeds (0.0557 day^{-1}) was significantly faster ($P < 0.05$) than from carboxin + thiram treated seeds (0.0512 day^{-1}) only in wet soil. Freezing at seven and 14 days significantly reduced final emergence after planting ($P < 0.05$). Final emergence from carboxin + thiram treated seeds (78 %) was significantly above ($P < 0.05$) untreated seeds (59 %). Final emergence was significantly increased ($P < 0.05$) from dry soil (72 %) compared to wet soil (66 %). No correlations occurred between rate of emergence and final emergence.

Carboxin + thiram treatment (27 %) significantly reduced ($P < 0.05$) seedling disease index compared to untreated seeds (55 %). Seedlings grown in dry soil (48 %) had significantly increased ($P < 0.05$) seedling disease index above seedlings grown in wet soil (34 %). No correlations occurred between rate of emergence and disease severity. Seedlings grown in dry soil (34.7 mg) had significantly increased ($P < 0.05$) dry weight above seedlings grown in wet soil (32.6 mg).

Freezing at 0 °C for 24 h, seven, 14 and 21 days after planting significantly reduced ($P < 0.05$) rate of seedling emergence from seedlot 10 (cv. Riband; 73 % infection). Freezing seven days after planting reduced rate of emergence most severely ($P < 0.05$). Carboxin + thiram treatment (0.0597 day^{-1}) significantly reduced rate of emergence ($P < 0.05$) below untreated seeds (0.0613 day^{-1}). Rate of emergence from dry soil (0.0575 day^{-1}) was significantly slower ($P < 0.05$) than from wet soil (0.0636 day^{-1}). Rate

Table 22. Effect of timing of 24 h freezing (0 °C) on rate of emergence (day^{-1}), final emergence, disease index and dry weight (mg) of winter wheat seedlings from a seedlot (cv. Equinox) with 88 % *Microdochium nivale* infection.

freezing timing*	soil water content (% w/w)	rate of emergence	% emergence	disease index		seedling dry wt
untreated seeds						
none	20	0.0471	69	64	54**	34.2
none	40	0.0563	61	48	44	31.7
7	20	0.0451	61	57	49	33.1
7	40	0.0539	46	63	53	32.9
14	20	0.0464	56	54	47	36.7
14	40	0.0573	49	51	47	28.8
21	20	0.0463	61	59	50	34.1
21	40	0.0545	62	46	41	34.9
28	20	0.0457	62	57	49	33.5
28	40	0.0564	65	55	50	31.6
carboxin + thiram treated seeds						
none	20	0.0478	84	34	35	37.3
none	40	0.0536	84	18	20	33.8
7	20	0.0444	83	45	41	33.4
7	40	0.0495	69	15	17	31.0
14	20	0.0435	82	37	37	32.5
14	40	0.0503	70	13	17	32.5
21	20	0.0479	81	35	36	37.7
21	40	0.0502	77	8	12	34.1
28	20	0.0486	77	42	40	34.9
28	40	0.0525	77	24	27	34.5
LSD ($P < 0.05$)		0.00345	13.9	-	11.5	4.66
SED		0.00175	7.0	-	5.8	2.36
DF		179	179	-	179	179
%cv		7.8	22.9	-	34.0	15.7

* days after planting.
** data angular transformed.
Seedlot **6** (cv. Equinox; 88 % infection) see Table 2; page 33.

of emergence was significantly faster ($P < 0.05$) from wet soil above dry soil for all freezing timings except for freezing seven days after planting (Table 23). The seed treatment x soil water content interaction was significant ($P < 0.001$) for final emergence. In wet soil, final emergence from carboxin + thiram treated seeds (58 %) was significantly below untreated seeds (68 %) but the opposite occurred in dry soil (carboxin + thiram

treated seeds 89 %: untreated seeds 81 %). There was no correlation between rate of emergence and final emergence.

Seedling disease index could not be statistically analysed due to a lack of normality. Seedling disease index was reduced by carboxin + thiram treatment. No correlations occurred between rate of emergence and disease index. Freezing at 0 ^{0}C for 24 h significantly increased seedling dry weight ($P < 0.05$). Carboxin + thiram treatment (27.2 mg) significantly increased ($P < 0.05$) seedling dry weight above seedling from untreated seeds (25.1 mg). Seedlings grown in wet soil (26.9 mg) were significantly heavier ($P < 0.05$) than seedlings from dry soil (25.4 mg).

Effect of freezing for 12 h at -5 ^{0}C and carboxin + thiram seed treatment on seedling growth and seedling blight severity from seed-borne *Microdochium nivale* infection

For seedlot 2 (cv. Cadenza; 29 % infection), rate of emergence was significantly reduced by freezing seven days after planting ($P < 0.001$). Rate of emergence was significantly ($P < 0.001$) faster from wet soil (0.1072 day^{-1}) than dry soil (0.0839 day^{-1}). The seed treatment x soil water interaction was significant ($P = 0.010$). Rate of emergence from carboxin + thiram treated seeds was significantly ($P < 0.05$) faster than from untreated seeds only in dry soil (Table 24). Freezing seven days after planting significantly reduced final emergence ($P < 0.05$). Carboxin + thiram seed treatment significantly ($P < 0.05$) increased final emergence (89 %) above untreated seeds (77 %). There was no correlation between rate of emergence and final emergence.

Table 23. Effect of timing of 24 h freezing (0 °C) on rate of emergence (day^{-1}), final emergence, disease index and dry weight (mg) of winter wheat seedlings from a seedlot (cv. Riband) with 73 % *Microdochium nivale* infection.

freezing timing*	soil water content (% w/w)	rate of emergence	% emergence		disease index		seedling dry wt
untreated seeds							
none	20	0.0602	77	62**	21	(6.0)	23.6
none	40	0.0690	72	61	16	(5.0)	22.6
7	20	0.0577	82	69	9	(3.7)	23.9
7	40	0.0592	68	58	10	(5.0)	24.4
14	20	0.0584	80	64	10	(4.3)	26.4
14	40	0.0596	71	59	5	(2.1)	27.5
21	20	0.0554	77	62	13	(3.7)	25.6
21	40	0.0656	64	54	15	(5.2)	26.2
28	20	0.0615	90	76	10	(4.0)	23.0
28	40	0.0665	64	55	13	(9.7)	27.3
carboxin + thiram treated seeds							
none	20	0.0577	88	73	2	(1.1)	26.1
none	40	0.0664	53	47	0	(0.0)	24.0
7	20	0.0566	92	78	0	(0.0)	25.2
7	40	0.0593	62	52	5	(2.8)	26.1
14	20	0.0542	86	71	3	(1.9)	27.5
14	40	0.0630	59	50	0	(0.0)	30.0
21	20	0.0575	88	71	0	(0.0)	26.4
21	40	0.0642	53	47	16	(9.8)	26.5
28	20	0.0556	89	74	1	(1.0)	26.4
28	40	0.0634	63	53	0	(0.0)	34.1
LSD ($P < 0.05$)		0.00476	-	10.2	-		4.01
SED		0.00241	-	5.2	-		2.03
DF		179	-	179	-		179
%cv		8.9	-	18.7	-		17.4

* days after planting.
Numbers in parentheses represent SE.
Seedlot **10** (cv. Riband; 73 % infection) see Table 2; page 33.

Table 24. Effect of timing of 12 h freezing (-5 °C) on rate of emergence (day^{-1}), final emergence, disease index and dry weight (mg) of winter wheat seedlings from a seedlot (cv. Cadenza) with 29 % *Microdochium nivale* infection.

freezing timing*	soil water content (% w/w)	rate of emergence		% emergence	disease index		seedling dry wt	
untreated seeds								
none	20	0.0860	0.293[1]	85	42	40[2]	28.0	3.33[3]
none	40	0.1107	0.332	91	75	62	24.9	3.19
7	20	0.0685	0.258	45	72	61	31.5	3.40
7	40	0.0966	0.310	33	86	69	20.2	2.90
14	20	0.0860	0.293	81	93	78	13.1	2.37
14	40	0.1113	0.333	86	87	71	10.7	2.33
21	20	0.0854	0.291	88	81	66	17.4	2.84
21	40	0.1103	0.331	89	85	70	14.3	2.57
28	20	0.0835	0.288	88	78	62	20.5	3.00
28	40	0.1104	0.331	86	81	65	19.8	2.96
carboxin + thiram treated seeds								
none	20	0.0880	0.296	96	12	18	27.5	3.31
none	40	0.1093	0.331	96	55	48	28.2	3.31
7	20	0.0750	0.271	69	41	34	29.6	3.38
7	40	0.0932	0.304	47	89	77	20.3	2.94
14	20	0.0899	0.300	100	85	72	16.0	2.68
14	40	0.1085	0.329	96	92	77	11.3	2.32
21	20	0.0870	0.295	97	79	63	17.6	2.84
21	40	0.1118	0.334	96	83	67	13.7	2.55
28	20	0.0899	0.299	95	71	59	21.0	3.03
28	40	0.1097	0.331	97	71	58	21.9	3.03
LSD ($P < 0.05$)		-	0.0115	16.6	-	12.4	-	0.298
SED		-	0.0058	8.4	-	6.3	-	0.151
DF		-	179	179	-	179	-	179
%cv		-	4.2	22.6	-	23.2	-	11.6

* days after planting.
1 data square root transformed.
2 data angular transformed.
3 data natural logarithm transformed.
Seedlot **2** (cv. Cadenza; 29 % infection) see Table 2; page 33.

Freezing significantly increased the seedling disease index ($P < 0.05$). Greatest seedling disease index occurred on seedlings frozen 14 days after planting ($P < 0.05$). The disease index on seedlings grown in wet soil (80 %) was significantly higher ($P < 0.05$) than seedlings grown in dry soil (65 %). The timing of freezing x seed treatment interaction

was significant (P = 0.049). Carboxin + thiram seed treatment significantly reduced seedling disease index only on seedlings not exposed to -5 °C ($P < 0.05$). The freezing timing x soil water content interaction was significant ($P < 0.001$). Disease index on seedlings growing in wet soil was significantly greater ($P < 0.05$) than seedlings growing in dry soil only for seedlings not frozen and frozen for -5 °C for 12 h seven days after planting. The seed treatment x soil water content interaction was significant ($P = 0.014$). Carboxin + thiram seed treatment significantly reduced ($P = 0.014$) seedling disease index (58 %) below untreated seeds only in dry soil (73 %). The timing of freezing x seed treatment x soil water content was significant ($P = 0.030$). Significant differences ($P < 0.05$) only arose for seedlings not frozen and exposed to freezing seven days after planting. Timing of freezing significantly affected seedling dry weight ($P < 0.001$). Freezing at seven days caused severest reductions in seedling dry weight ($P < 0.05$). Seedlings growing in dry soil were significantly heavier than seedlings growing in wet soil for all freezing timings ($P < 0.05$). Carboxin + thiram had an inconsistent effect on seedling dry weight.

Twelve hours freezing at -5 °C, seven and 14 days after planting significantly reduced ($P < 0.05$) rate of emergence from seedlot 6 (cv. Equinox; 88 % infection). Rate of emergence was most severely ($P < 0.05$) affected by freezing seven days after planting. Seedlings emerged significantly faster ($P < 0.05$) from wet soil (0.0537 day^{-1}) than from dry soil (0.0497 day^{-1}). Final emergence could not be statistically analysed due to a lack of normality. All freezing timings typically reduced final emergence from untreated seeds (Table 25). Seven and 14 day freezing timings severely reduced final emergence from treated and untreated seeds. Final emergence from treated seeds was typically greater than from untreated seeds. There was no correlation between rate of emergence and final emergence. Seedling disease index could not be statistically analysed due to a lack of

Table 25. Effect of timing of 12 h freezing (-5 °C) on rate of emergence (day^{-1}), final emergence, disease index and dry weight (mg) of winter wheat seedlings from a seedlot (cv. Equinox) with 88 % *Microdochium nivale* infection.

freezing timing*	soil water content (%w/w)	rate of emergence	% emergence		disease index		seedling dry wt
untreated seeds							
none	20	0.0513	71	(4.3)	67	(5.0)	31.5
none	40	0.0587	68	(3.6)	76	(3.3)	30.0
7	20	0.0449	49	(9.5)	79	(6.1)	32.5
7	40	0.0546	55	(8.9)	78	(4.9)	28.9
14	20	0.0483	20	(9.9)	91	(5.4)	42.3
14	40	0.0568	36	(11.7)	93	(3.1)	27.4
21	20	0.0502	61	(5.5)	88	(4.6)	31.0
21	40	0.0597	65	(4.3)	92	(4.0)	26.5
28	20	0.0509	57	(3.0)	84	(4.8)	30.2
28	40	0.0613	76	(3.4)	77	(6.7)	27.6
carboxin + thiram treated seeds							
none	20	0.0526	82	(3.6)	23	(7.8)	30.9
none	40	0.0610	77	(3.7)	26	(8.0)	34.0
7	20	0.0463	60	(12.0)	42	(13.9)	31.9
7	40	0.0489	51	(10.5)	61	(14.1)	33.7
14	20	0.0478	42	(13.0)	54	(12.1)	29.5
14	40	0.0577	53	(12.1)	77	(8.5)	29.6
21	20	0.0520	84	(4.0)	51	(10.0)	32.4
21	40	0.0622	84	(3.4)	73	(3.8)	29.5
28	20	0.0532	82	(4.2)	53	(9.0)	30.0
28	40	0.0621	80	(4.7)	32	(8.8)	31.1
LSD ($P < 0.05$)		0.00395	-		-		4.69
SED		0.00200	-		-		2.38
DF		179	-		-		179
%cv		8.3	-		-		17.1

* days after planting.
Numbers in parentheses represent SE.
Seedlot **6** (cv. Equinox; 88 % infection) see Table 2; page 33.

normality. All freezing timings increased disease index on seedlings from treated and untreated seeds. Carboxin + thiram treatment greatly reduced the disease index. Higher disease index occurred on seedlings grown in wet soil. No correlations occurred between rate of emergence and disease index. Seedlings from dry soil (32.2 mg) were significantly heavier ($P < 0.05$) than seedlings from wet soil (29.8 mg). The timing of freezing x seed

treatment interaction was significant (P = 0.007). Seedlings from carboxin + thiram treated seeds were significantly ($P < 0.05$) heavier than from untreated seeds across all freezing timings except for freezing 14 days after planting. The timing of freezing x soil water interaction was significant (P = 0.006). Seedlings grown in dry soil were significantly heavier ($P < 0.05$) than seedlings grown in wet soil exposed to freezing 14 and 21 days after planting. The seed treatment x soil water interaction was significant ($P < 0.001$). In wet soil, seedlings from carboxin + thiram treated seeds (31.6 mg) were significantly heavier ($P < 0.05$) than seedlings from untreated seeds (28.1 mg). In dry soil, seedlings from untreated seeds (33.5 mg) were significantly heavier ($P < 0.05$) than seedlings from carboxin + thiram treated seeds (31.0 mg).

Effect of freezing for 24 h at -5 °C and carboxin + thiram seed treatment on seedling growth and seedling blight severity from seed-borne *Microdochium nivale* infection

Twenty four hours freezing to -5 °C, seven days after planting significantly reduced ($P < 0.05$) rate of emergence from seedlot 2 (cv. Cadenza; 29 % infection). Seedlings from carboxin + thiram treated seeds (0.0890 day^{-1}) emerged significantly faster ($P < 0.05$) than untreated seeds (0.0854 day^{-1}). Seedlings growing in wet soil (0.0996 day^{-1}) emerged significantly faster ($P < 0.05$) than seedlings growing in dry soil (0.0749 day^{-1}) across all freezing timings. The timing of freezing x seed treatment x soil water interaction was significant (Table 26).

Final emergence was significantly reduced by 24 h freezing at -5 °C, seven and 14 days after planting ($P < 0.05$). Freezing seven days after planting most severely ($P < 0.05$) reduced final emergence. Carboxin + thiram treatment significantly increased ($P < 0.05$) final emergence (91 %) above untreated seeds (82 %). However final emergence from treated seeds was only significantly increased ($P \leq 0.05$) in dry soil. There was no correlation between rate of emergence and final emergence.

Table 26. Effect of timing of 24 h freezing (-5 °C) on rate of emergence (day^{-1}), final emergence, disease index and dry weight (mg) of winter wheat seedlings from a seedlot (cv. Cadenza) with 29 % *Microdochium nivale* infection.

freezing timing*	soil water content (% w/w)	rate of emergence		% emergence		disease index	seedling dry wt	
untreated seeds								
none	20	0.0792	0.281[1]	83	49	44[2]	25.6	3.24[3]
none	40	0.1014	0.318	93	76	61	27.7	3.30
7	20	0.0635	0.251	60	62	55	25.5	3.21
7	40	0.0844	0.290	68	79	66	22.2	3.05
14	20	0.0767	0.277	74	93	78	18.0	2.66
14	40	0.1013	0.318	86	95	81	8.3	2.02
21	20	0.0781	0.279	88	80	63	16.3	2.77
21	40	0.1035	0.321	89	84	67	13.7	2.57
28	20	0.0741	0.272	88	67	55	20.9	3.03
28	40	0.0999	0.316	89	75	60	20.1	2.96
carboxin + thiram treated seeds								
none	20	0.0789	0.281	95	14	20	29.0	3.36
none	40	0.1077	0.328	98	61	51	31.4	3.39
7	20	0.0699	0.264	91	33	32	27.0	3.28
7	40	0.0814	0.284	58	79	69	23.6	3.13
14	20	0.0783	0.280	91	88	75	19.4	2.90
14	40	0.1087	0.330	95	94	80	8.6	2.10
21	20	0.0800	0.283	94	81	64	15.9	2.73
21	40	0.1064	0.326	93	83	67	15.0	2.69
28	20	0.0780	0.279	97	70	57	24.3	3.17
28	40	0.1059	0.325	94	75	60	20.1	2.96
LSD ($P < 0.05$)		-	0.0103	14.6	-	11.2	-	0.277
SED		-	0.0052	7.4	-	5.7	-	0.141
DF		-	179	179	-	179	-	179
%cv		-	3.9	19.1	-	21.1	-	10.7

* days after planting.
1 data square root transformed.
2 data angular transformed.
3 data natural logarithm transformed.
Seedlot **2** (cv. Cadenza; 29 % infection) see Table 2; page 33.

Disease severity was significantly increased ($P < 0.05$) by 24 h freezing at -5 °C. Disease severity was most severe following freezing 14 days after planting ($P < 0.05$). Carboxin + thiram seed treatment (68 %) significantly reduced ($P < 0.05$) the disease index compared to untreated seeds (76 %). Seedlings growing in wet soil (80 %) had significantly

increased ($P < 0.05$) disease index compared to seedlings growing in dry soil (64 %). Carboxin + thiram seed treatment significantly reduced ($P < 0.05$) the disease index on seedlings or frozen seven days after planting. Seedlings grown in wet soil were more diseased than seedlings grown in dry soil across all freezing timings. However, significant differences ($P < 0.05$) occurred only for seedlings not frozen or frozen at seven days after planting. Seedlings from untreated seeds were more diseased than seedlings from carboxin + thiram treated seeds grown in wet and dry soil. However, this difference was only significant in dry soil ($P < 0.05$). No correlations occurred between rate of emergence and disease index.

Seedling dry weight was significantly reduced by 24 h freezing at -5 °C ($P < 0.001$). Freezing 14 days after planting caused most severe reductions in seedling dry weight ($P < 0.05$). Seedlings grown in dry soil (22.2 mg) were significantly heavier ($P < 0.05$) than seedlings grown in wet soil (19.1 mg). Seedling dry weight was significantly increased ($P < 0.05$) by carboxin + thiram seed treatment (21.4 mg) above untreated seeds (19.8 mg). Seedlings grown in dry soil were significantly heavier ($P < 0.05$) than seedlings grown in dry soil when exposed to -5 °C for 24 h.

Freezing seven and 14 days after planting significantly ($P < 0.05$) reduced rate of emergence from seedlot 6 (cv. Equinox; 88 % infection). Seedlings from wet soil (0.0572 day^{-1}) emerged significantly faster ($P < 0.05$) than seedlings from dry soil (0.0513 day^{-1}). Final emergence was significantly reduced ($P < 0.05$) by 24 h freezing to -5 °C, seven and 14 days after planting (Table 27). Final emergence from carboxin + thiram treated seeds (74 %) was significantly greater ($P < 0.05$) than from untreated seeds (58 %). There was no correlation between rate of emergence and final emergence.

Table 27. Effect of timing of 24 h freezing (-5 °C) on rate of emergence (day^{-1}), final emergence, disease index and dry weight (mg) of seedlings from a winter wheat seedlot (cv. Equinox) with 88 % *Microdochium nivale* infection.

freezing timing*	soil water content (% w/w)	rate of emergence		% emergence		disease index		seedling dry wt
untreated								
none	20	0.0536	-2.94[1]	76	61[2]	66	(2.2)	31.5
none	40	0.0593	-2.83	73	59	72	(2.8)	29.6
7	20	0.0452	-3.11	48	44	84	(4.9)	34.2
7	40	0.0483	-3.05	32	31	92	(3.5)	29.0
14	20	0.0459	-3.12	37	35	83	(7.0)	32.2
14	40	0.0554	-2.92	37	34	99	(1.1)	24.0
21	20	0.0532	-2.95	66	55	96	(2.5)	31.7
21	40	0.0598	-2.83	72	59	95	(2.5)	26.0
28	20	0.0545	-2.92	73	61	77	(5.1)	34.2
28	40	0.0593	-2.84	68	56	87	(3.3)	28.0
carboxin + thiram treated								
none	20	0.0561	-2.90	86	74	22	(7.5)	32.7
none	40	0.0602	-2.82	85	70	29	(9.6)	31.0
7	20	0.0476	-3.06	76	63	37	(12.0)	32.5
7	40	0.0494	-3.02	66	56	64	(11.2)	31.9
14	20	0.0469	-3.09	48	44	64	(8.9)	34.5
14	40	0.0558	-2.92	48	43	85	(5.9)	29.5
21	20	0.0542	-2.93	82	67	61	(9.6)	30.7
21	40	0.0593	-2.84	86	71	66	(10.7)	28.5
28	20	0.0545	-2.92	78	65	58	(3.5)	31.8
28	40	0.0617	-2.80	88	73	65	(6.5)	29.6
LSD ($P < 0.05$)		-	0.071	-	13.7	-		5.19
SED		-	0.036	-	6.9	-		2.63
DF		-	179	-	179	-		179
%cv		-	2.7	-	27.7	-		19.2

* days after planting
1 data natural logarithm transformed.
2 data angular transformed.
Numbers in parentheses represent SE.
Seedlot **6** (cv. Equinox; 88 % infection) see Table 2; page 33.

Disease index could not be statistically analysed due to a lack of normality. Freezing increased the disease index on all seedlings. Carboxin + thiram seed treatment greatly reduced the disease index in wet and dry soil compared to untreated seeds. The disease index was increased on seedlings grown in wet soil. No correlations occurred between rate

of emergence and disease index. Twenty-four h freezing at -5 °C typically reduced seedling dry weight from treated seeds but had an inconsistent effect on seedlings from untreated seeds. There was no consistent effect for carboxin + thiram seed treatment on seedling dry weight. Seedlings growing in dry soil (32.6 mg) were significantly heavier ($P < 0.05$) than seedlings growing in wet soil (28.7 mg).

DISCUSSION

This is the first reported work showing that timing of freezing and its severity can differentially affect seedling growth and seedling blight disease from *M. nivale* infected seeds. Pre-emergent freezing (-5 °C, seven days after planting) had the most severe effect on final emergence and disease severity. Differences were observed between freezing severities. Exposure to -5 °C for 12 and 24 h slowed rate of emergence and final emergence and typically reduced seedling growth and increased seedling blight. Exposure to 0 °C for 12 and 24 h also slowed rate of emergence but had an inconsistent effect on emergence and no consistent effect on seedling growth and seedling blight. Freezing at -5 °C appeared to have the most severe effects on seedling growth whilst 0 °C did not severely affect seedling growth. This may be because freezing to -5 °C reduced more severely seedling growth giving *M. nivale* an increased opportunity for infection. However, the experimental design means that direct comparisons of 0 and -5 °C is not possible. Pre-emergent freezing (0 °C for 12 h on four consecutive nights) reduced perennial ryegrass root length, shoot height and fresh weight in soil containing *M. nivale* mycelium (Holmes & Channon, 1975). One, two or three freezing periods immediately after inoculation did not increase seedling damage compared to seedlings not frozen. Hare *et al.* (1995) described a strong correlation between rate of emergence and final emergence under constant temperatures for a winter wheat seedlot (cv. Riband) with 72 % *M. nivale*

infection. The lack of such a correlation in this investigation may imply freezing was disrupting the host-pathogen interaction occurring under controlled temperature regimes.

Duration of freezing did not appear to increase seedling blight severity, measured by reduced final emergence and increased disease index. This implies that freezing to -5 °C rather than duration of freezing -as shown in this study- is the important determinant of disease. It is unknown whether more severe freezing increases *M. nivale* pathogenicity or pre-disposes seedlings to infection. However, the experimental design means that direct comparisons of 12 and 24 h freezing duration is not possible. Freezing increased the incidence of *M. nivale* isolation from oat seedling mesocotyls and roots from untreated pathogen-free seeds in a controlled environment investigation (Rawlinson & Colhoun, 1970). Freezing for 48 h at -2 °C also increased the incidence of *M. nivale* isolation from spring barley seedling coleoptiles growing from surface-inoculated seeds (Perry, 1986).

Investigations conducted by additional researchers have supported the hypothesis that freezing pre-disposes seedlings to attack, possibly by creating sites of dead tissue, facilitating pathogen entry. The recovery and survival of barley (cv. Hudson) after freezing for 1 h at -10 °C was reduced by soil-borne *F. avenaceum* inoculation (Smith & Olien, 1978). Bailey *et al.* (1982) presented evidence that wheat (cv. Genesee) root exudate caused by freezing, promoted *Cephalosporium gramineum* conidia germination and germ-tube extension, resulting in root disease. Soil freezing (up to 96 h at -1 °C) caused slight necrosis and increased disease severity on white lupins from soil-borne *Botrytis cinerea*, *F. aveaneacum* and *Pleiochaeta setosa* inoculum (Etheridge & Bateman, 1998). However, it is likely that different sources of inoculum and pathogens will react differently to freezing. The above investigations imply freezing effects were due entirely to deleterious effects on the seedling.

However, additional researchers have demonstrated freezing to have little effect on winter wheat seedlings free from pathogen attack. Seventy-two hours freezing at -6 °C caused no visible injuries on winter wheat (cv. Frederick) seedlings (Pomeroy *et al.*, 1985). Tanino & McKersie (1985) described a brief but not specified freezing at -8 °C. having no effect on winter wheat (cv. Frederick) regrowth or viability of the vascular transition zone and apical meristem. Windt & van Hasselt (1999) also described winter wheat (cv. Urban) seedlings surviving overnight freezing at -11 °C. These investigations imply that *M. nivale* pathogenicity is increased at -5 °C, rather than seedling death caused by freezing. At this time, *M. nivale* responses to sub-zero temperatures are undocumented. In addition, the hypothesis that -5 °C is facilitating *M. nivale* entry by producing dead tissue needs to be tested.

Differences between the seedlots used in this investigation were observed, although direct comparisons are not possible because they were tested in different experiments. This may be due to differences in cultivar responses to freezing, or differences in the depth and intensity of *M. nivale* seed infection. The effect of depth of *M. nivale* seed infection on seedling blight is an avenue for further research. Freezing generally affected seedlings more severely when they were grown in dry soil, probably through reduced seedling growth. *Microdochium nivale* seedling blight has been documented to be more severe in dry soils (Hare, 1997). In this investigation, rate of emergence was generally faster from seeds growing in wet soil than dry soil for freezing at 0 and -5 °C for 12 and 24 h. However increased final emergence and increased seedling growth did not occur from wet soil and seedlings were more diseased than those growing in dry soil. Reasons for this are unknown. Freezing effects may also be exaggerated on seedlings with reduced vigour. There was no evidence from the results in this investigation that seedling vigour, measured by seedling dry weight, was related to the germination potential of the three seedlots used.

This investigation demonstrated an effect of the severity of freezing on the performance of carboxin + thiram seed treatment. However, duration of freezing appeared to have no effect on carboxin + thiram performance. Carboxin + thiram treatment appeared to be more effective, measured by final emergence and reduced disease severity, after freezing at 0 than -5 °C. Carboxin + thiram seed treatment did not significantly increase seedling growth. Very few investigations have been conducted into the effect of freezing on seed treatment performance. Rawlinson & Colhoun (1970) proposed organomercury seed treatment increased seedling vigour from a pathogen-free oat seedlot under conditions including frost, by protecting the mesocotyls from soil-borne fungi. However, it was not clear whether the organomercury treatment increased seedling vigour or reduced fungal attack, since unsterilised soil was used. However, triadimenol, prochloraz, imazalil and carboxin seed treatments decreased the freezing tolerance of winter wheat (cv. Norstar) crowns and had no beneficial effect on winter wheat (cv. Norwin) (Gusta *et al.*, 1994).

CHAPTER 7

Effects of seedling blight resulting from seed-borne *Microdochium nivale* infection and of carboxin + thiram seed treatment on foot rot disease incidence, stem colonisation, subsequent plant growth and yield

INTRODUCTION

Microdochium nivale is a widespread pathogen on winter wheat stem-bases in the UK. In a survey of stem-bases obtained from 339 winter wheat crops in Scotland between 1970-4, *M. nivale* was isolated from 15 to 25 % of stem-bases (Rennie *et al.*, 1983). In 1986, 82 % of *Fusarium* pathogens isolated from stem-bases of 375 winter wheat crops in England and Wales at GS 73-75 were *M. nivale* (Locke *et al.*, 1987). *Microdochium nivale* was the predominant *Fusarium* pathogen isolated from stem-bases of nine winter wheat crops in the Midlands between 1987-9 (Parry, 1990). In 1992, Pettitt *et al.* (1993) isolated *M. nivale* from 34 % of wheat stem-bases collected from around the UK. *Microdochium nivale* was also the predominant stem-base pathogen at GS 31 and GS 73-75 in 1989 but not 1990 in UK surveys conducted by Polley & Turner (1995). However, in none of these investigations was the source of inoculum determined.

Little work has been undertaken to determine the effects of seed-borne *M. nivale* infection on wheat growth after GS 12. Millar & Colhoun (1969a) described reduced incidence of tillering and fewer shoots plant^{-1} from winter wheat (cv. Viking) seeds inoculated with 1000 spores ml^{-1}. In contrast, much research has been published on *Fusarium* foot rot. However, a link between non-lethal *M. nivale* seedling blight and foot rot remains unproven. Several workers have demonstrated that foot rot caused by *F. culmorum*, *F. graminearum* and *M. nivale* can lead to subsequent stem colonisation (Snijders, 1990; Hutcheon & Jordan, 1992; Clement & Parry, 1998). However, no investigations have used seed-borne inoculum, which can manifest itself on the seedling coleoptile under favourable conditions for *M. nivale* disease development. Therefore, the potential for seed-borne *M. nivale* to cause foot rot and subsequent stem colonisation remains undefined.

There is little evidence that seed treatments can reduce *Fusarium* foot rot disease. Perry (1986) recorded stem-base infections more frequently on seedlings from untreated seeds than from triadimenol + fuberidazole treated seeds in field trials in the UK in 1982 and 1983 with spring barley (cv. Golden Promise) seeds, surface-inoculated with *M. nivale* spores. *Microdochium nivale* from naturally infected and surface-inoculated seeds probably behaves very differently around the stem-base. Indeed, Hare (1997) using seeds naturally infected with *M. nivale*, observed that foot rot incidence was typically greater on plants grown from treated seeds, than those grown from untreated seeds at GS 45 and GS 75 during two field trials in the UK in 1994. This is possibly because the infected seedlings from untreated seeds had died. In six field trials in 1992-3 in the UK, fludioxonil and guazatine provided 81 and 62 % control respectively of stem-base browning, 109-208 days after planting of winter wheat seedlots (cv. Riband and cv. Slejpner) with 70 and 71 % *M. nivale* infection respectively (West *et al.*, 2001).

In many studies investigating the performance of seed treatments against seedling blight, inoculum was not seed-borne which is the major source of *M. nivale* infection in the UK. In addition, inoculum amounts were probably above those typically found under field conditions. The aims of the work described in this chapter were to (i) determine a possible link between seedling blight caused by seed-borne *M. nivale* infection and foot rot. (ii) establish the extent of stem colonisation from seed-borne *M. nivale* infection. (iii) examine the effect of seedling blight severity on foot rot disease incidence, stem colonisation, subsequent plant growth and yield. (iv) determine the effect of carboxin + thiram seed treatment on foot rot disease incidence, stem colonisation, subsequent plant growth and yield through four glasshouse trials.

MATERIALS & METHODS

Two experiments were designed to test the following hypotheses: (i) seedling blight does not lead to subsequent foot rot infection. (ii) seed-borne *M. nivale* infection has no effect on stem-colonisation. (iii) seedling blight disease severity has no effect on subsequent plant growth and (iv) carboxin + thiram treatment of infected seeds has no effect on subsequent plant growth and *M. nivale* stem colonisation.

Surface-sterilised seeds of seedlot 2 (cv. Cadenza; 29 % infection) (Table 2: page 33) with and without carboxin + thiram treatment, were planted into autoclaved John Innes No. 2 compost in sterilised seed trays (Chapter 3). Two experiments were conducted under 12 h light. In the first experiment, trays were incubated at 3 °C (cold incubation) to ensure disease expression. In the second experiment, trays were incubated at 22 °C (warm incubation) to prevent disease expression. At GS 12, seedlings were uprooted, washed and assessed for seedling blight (Table 28). Removing the seed, the coleoptile, or the coleoptile and seed of seedlings from treated seeds or seedlings with disease scores one, two and three produced the experimental treatments (Table 29). The seed and coleoptile were removed because these are considered to be the major source of *M. nivale* inoculum during seedling growth. No disease symptoms were visible beneath the coleoptile. Isolations taken from coleoptile lesions confirmed *M. nivale* was the causal agent of disease.

Table 28. Assessment of seedling blight severity from seed-borne *Microdochium nivale* infection.

disease score	description
0	no visible symptoms on roots or coleoptile
1	slight root and coleoptile necrosis
2	moderate root and coleoptile necrosis
3	severe root and coleoptile necrosis and plant stunting

Five seedlings of each treatment were planted into 1.5 kg autoclaved John Innes No. 2 compost in surface-sterilised pots (15 cm diameter x 14 cm height: Sankey, Colne, Lancashire, UK). Pots were placed in a glasshouse (12 h 15 °C / 12h 5 °C) in a randomised design with ten blocks with 12 h light day^{-1}. Pots were watered daily and nicotine shreds (Dow AgroSciences, Hitchin, Hertfordshire, UK) and fenpropimorph (BASF, Bury St. Edmunds, UK) applied as necessary, to control aphid and powdery mildew infestations respectively. To each pot, 0.5 g Nitram fertiliser (34.5 % N: Kemira Agro UK Ltd, Chester, Cheshire, UK) was applied at GS 30. Each experiment was repeated twice.

Table 29. Experimental treatments for an investigation into the effect of *Microdochium nivale* seedling blight severity and carboxin + thiram seed treatment on subsequent plant vigour, productivity, foot rot incidence and stem colonisation.

tre	temp[1]	treatment[2]	disease score[3]	seedling components removed
1	COLD	+	0	none
2	COLD	+	0	seed
3	COLD	–	1	none
4	COLD	–	1	seed
5	COLD	–	1	coleoptile
6	COLD	–	1	seed + coleoptile
7	COLD	–	2	none
8	COLD	–	2	seed
9	COLD	–	2	coleoptile
10	COLD	–	2	seed + coleoptile
11	COLD	–	3	none
12	COLD	–	3	seed
13	COLD	–	3	coleoptile
14	COLD	–	3	seed + coleoptile
15	WARM	+	0	none
16	WARM	+	0	seed
17	WARM	–	0	none
18	WARM	–	0	seed

1 COLD = seedlings grown at 3 °C, WARM = seedlings grown at 22 °C.
2 + = carboxin + thiram treated seeds, – = untreated seeds.
3 assessed according to Table 28.

Seedling establishment (GS 15-25), shoot number (GS 39) and ear number (GS 75) were recorded. At GS 40-49, one plant per pot was harvested and washed under running water. Main stem length (stem-base to flag leaf ligule) was measured and leaf area determined using WINDIAS 2.0 (Delta-T Devices Ltd, Cambs, UK). A 20 mm segment of tissue was removed from the stem-base and each node, surface-sterilised, plated onto PDA amended with 130 µg streptomycin sulfate ml^{-1} agar and 25 µg carbendazim ml^{-1} agar and incubated in darkness at 15 °C (Chapter 3). Tissue segments were assessed for *M. nivale* presence after 7-10 days. The remainder of the plant was dried at 102 °C for 24 h and its dry weight determined. At harvest, % plant survival (GS 15-25 to harvest) was recorded. Yield at 15 % moisture, TGW, grains ear^{-1}, grain weight ear^{-1}, grains $plant^{-1}$ and grain weight $plant^{-1}$ were determined. Isolations were taken from the stem-base, nodes and ear of the main stem of one plant per pot.

The two seedling incubation temperatures were analysed separately. Data were analysed using ANOVA with experimental treatments (Table 29) as factors and seedling survival (GS 15-25), shoots $plant^{-1}$, stem length, leaf area and dry weight at GS 40-49, plant survival (GS 15-25 to harvest), ears $plant^{-1}$, yield, TGW, grains ear^{-1}, grain weight ear^{-1}, grains $plant^{-1}$ and grain weight $plant^{-1}$ as variables. Where appropriate, data were transformed to ensure normal distributions. Where data could not be ANOVA analysed due to lack of normality standard errors are presented. Foot rot disease incidence and stem colonisation are presented as % of plants infected.

RESULTS

Effect of seedling blight from seed-borne *Microdochium nivale* infection and carboxin + thiram seed treatment on plant productivity and yield

Seedling survival could not be statistically analysed due to a lack of normality. Seedling survival only declined for heavily diseased seedlings (Table 30). Shoots plant^{-1} declined as seedling blight severity increased but shoots plant^{-1} were only significantly reduced

Table 30. Effect of seedling blight severity, carboxin + thiram seed treatment and removal of seedling components on growth of winter wheat (cv. Cadenza) with seed-borne *Microdochium nivale* infection measured at GS 40-49 in pot trials after seedling growth at 3 °C.

treatment[1]	% survival	shoots plant^{-1}	leaf area (cm^2)		stem length (mm)	plant dry wt (g)	
1	99	2.96	172	4.90[2]	501	2.21	1.40[3]
2	100	3.00	128	4.67	468	1.67	1.23
3	100	3.09	122	4.64	460	1.65	1.22
4	100	3.04	154	4.82	480	1.69	1.21
5	99	2.87	114	4.56	476	1.79	1.27
6	100	2.83	134	4.66	489	1.68	1.23
7	100	2.90	150	4.82	490	2.07	1.35
8	100	2.70	141	4.60	464	1.64	1.20
9	100	2.85	121	4.51	461	1.51	1.14
10	99	2.78	137	4.69	473	1.82	1.29
11	91	2.65	136	4.69	432	1.73	1.21
12	89	2.50	111	4.42	441	1.58	1.16
13	94	2.55	106	4.40	441	1.29	1.08
14	97	2.35	113	4.37	414	1.06	0.95
LSD ($P < 0.05$)	-	0.330	-	0.269	40.1	-	0.178
SED	-	0.168	-	0.136	20.3	-	0.090
DF	-	245	-	245	245	-	245
^{0}ocv	-	19.0	-	9.3	13.9	-	23.6

1 **1** – seedlings from carboxin + thiram treated seeds; **2** – seeds removed from treatment 1; **3** – seedlings with disease score 1; **4** – seeds removed from treatment 3; **5** – coleoptiles removed from treatment 3; **6** – seeds and coleoptiles removed from treatment 3; **7** – seedlings with disease score 2; **8** – seeds removed from treatment 7; **9** – coleoptiles removed from treatment 7; **10** – seeds and coleoptiles removed from treatment 7; **11** – seedlings with disease score 3; **12** – seeds removed from treatment 11; **13** – coleoptiles removed from treatment 11; **14** – seeds and coleoptiles removed from treatment 11.

2 data natural logarithm transformed.

3 data square root transformed.

($P < 0.05$) from seedlings with a disease score of three (treatments 11-14). Plants from carboxin + thiram treated seeds had the greatest leaf area. Plants from seedlings with a disease score of three (treatments 11-14) had the least green leaf area ($P < 0.05$). Treatment one (carboxin + thiram treated seeds; no seedling components removed) produced the greatest plant dry weight at GS 40-49. Plants from seedlings with a disease score of three (treatments 11-14) had significantly less ($P < 0.05$) dry weight than plants from carboxin + thiram treated seeds (treatments 1 and 2). Heavily diseased seedlings (treatments 11-14) produced the shortest plants, measured by stem length ($P < 0.05$). Plants from carboxin + thiram treated seeds had the longest stem length.

Plant survival from GS 15-25 to harvest, could not be statistically analysed due to a lack of normality. Disease score and removal of seedling components had no effect on plant survival from GS 15-25 to harvest but plant survival from heavily diseased seedlings did decline (Table 31). Disease score and removal of the seedling components had no significant effect on ears plant^{-1} ($P = 0.762$) although seedling component removal did slightly increase ears plant^{-1} for heavily diseased seedlings (treatments 11-14). Ears plant^{-1} typically decreased with increased seedling disease. Plants from seedlings with a disease score of three (treatments 11-14) had significantly reduced ($P < 0.05$) yield and TGW. Removal of the seed and coleoptile typically decreased TGW, except for heavily diseased seedlings and treatment six (disease score one; seed and coleoptile removed). Ear productivity (ears plant^{-1} ($P = 0.762$), grains plant^{-1} ($P = 0.894$), grain weight plant^{-1} ($P = 0.439$), grains ear^{-1} ($P = 0.988$), grain weight ear^{-1} ($P = 0.786$)) were not significantly affected by seedling disease score or removal of the seed and coleoptile from seedlings.

Table 31. Effect of seedling blight severity, carboxin + thiram seed treatment and removal of seedling components on yield and its components of winter wheat (cv. Cadenza) with seed-borne *Microdochium nivale* in pot trials after seedling growth at 3 °C.

treatment[1]	% survival[2]	ears plant^{-1}	yield[3]	TGW (g)		grain wt (g) ear^{-1}		grains ear^{-1}	grains plant^{-1}	grain wt (g) plant^{-1}
1	100	2.98	15.7	44.3	6.61[4]	1.37	1.154[4]	31.6	93	4.02
2	100	3.03	15.8	42.7	6.49	1.32	1.142	31.3	94	3.94
3	100	3.07	16.7	44.7	6.64	1.41	1.181	32.3	97	4.18
4	100	3.04	16.0	42.8	6.51	1.34	1.150	32.1	97	4.00
5	96	3.01	15.9	43.7	6.55	1.41	1.173	32.4	97	4.14
6	99	2.90	15.9	47.3	6.85	1.43	1.184	30.0	85	4.00
7	100	2.95	16.7	46.6	6.79	1.43	1.189	31.1	92	4.17
8	99	2.81	14.7	45.5	6.70	1.34	1.146	29.8	86	3.76
9	100	2.79	15.2	44.7	6.64	1.40	1.172	31.7	88	3.81
10	99	2.92	14.3	42.2	6.46	1.24	1.107	30.3	89	3.66
11	94	2.68	12.0	41.9	6.43	1.31	1.134	31.6	83	3.38
12	89	3.05	12.9	43.4	6.55	1.34	1.148	31.3	94	3.93
13	91	2.90	12.3	41.1	6.37	1.29	1.131	32.1	91	3.60
14	87	2.79	12.4	42.5	6.46	1.33	1.135	31.8	89	3.58
LSD ($P < 0.05$)	-	NS	2.44	-	0.266	-	NS	NS	NS	NS
SED	-	0.199	1.24	-	0.135	-	0.0405	2.09	8.8	0.342
DF	-	245	245	-	245	-	245	245	245	245
%cv	-	21.6	26.5	-	6.5	-	11.1	21.1	30.5	27.9

1 **1** seedlings from carboxin + thiram treated seeds; **2** – seeds removed from treatment 1; **3** – seedlings with disease score 1; **4** seeds removed from treatment 3; **5** coleoptiles removed from treatment 3; **6** seeds and coleoptiles removed from treatment 3; **7** seedlings with disease score 2; **8** seeds removed from treatment **7**; **9** coleoptiles removed from treatment 7; **10** seeds and coleoptiles removed from treatment 7; **11** seedlings with disease score 3; **12** seeds removed from treatment **11**; **13** coleoptiles removed from treatment **11**; **14** seeds and coleoptiles removed from treatment 11.

2 from GS 15-25 to harvest

3 g pot^{-1}

4 data square root transformed.

Effect of seedling blight from seed-borne *Microdochium nivale* infection and carboxin + thiram seed treatment on foot rot disease incidence and stem colonisation

Data could not be analysed due to a lack of normality. No severe foot rot symptoms were observed on plants from seedlings grown at 3 °C. At GS 40-49, plants from all treatments were diseased at the stem-base and first node (Figure 7). There was slight *M. nivale* incidence on the second node for plants from all treatments except treatment six (disease score one; seed and coleoptile removed). Only for plants from treatment ten (disease score two; seed and coleoptile removed) was *M. nivale* detected on the third node at GS 40-49. Incidence of stem-base infections was greater than first node disease incidence across all treatments at GS 40-49. For all treatments, except eight (disease score two; seed removed) and ten (disease score two; seed and coleoptile removed), *M. nivale* incidence on the second node was less than on the first node. Seedling disease score had no consistent effect on the incidence of *M. nivale* on the stem-base, but plants from seedlings with a disease score of one (treatments 3-6) were typically less colonised than plants from all other treatments. There was no consistent correlation between *M. nivale* incidence on the stem-base and on the first node. Removal of seedling components had no effect on *M. nivale* colonisation of the stem.

At harvest, *M. nivale* was detected up to node four. *Microdochium nivale* incidence was typically increased at harvest compared to GS 40-49, especially on the first and second nodes. Carboxin + thiram seed treatment reduced *M. nivale* colonisation on and above the second node (Figure 8). *Microdochium nivale* was isolated from further up plants with increased seedling blight disease score, with plants from seedlings with disease score three (treatments 11-14) colonised up to the fourth node. Across all treatments, *M. nivale* incidence declined with increased node number. There was no correlation between *M. nivale* incidence at GS 40-49 and harvest or *M. nivale* on the stem-base and subsequent *M. nivale* incidence on plant nodes.

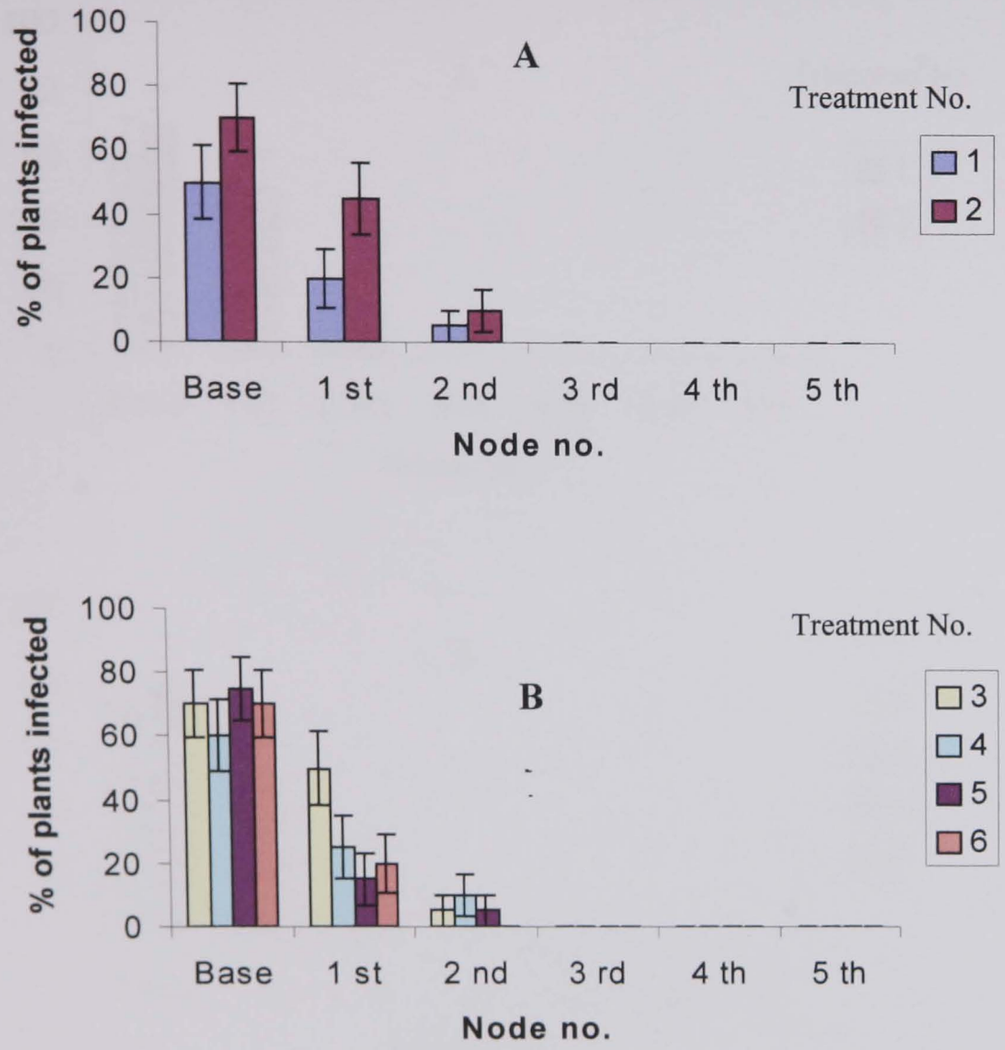
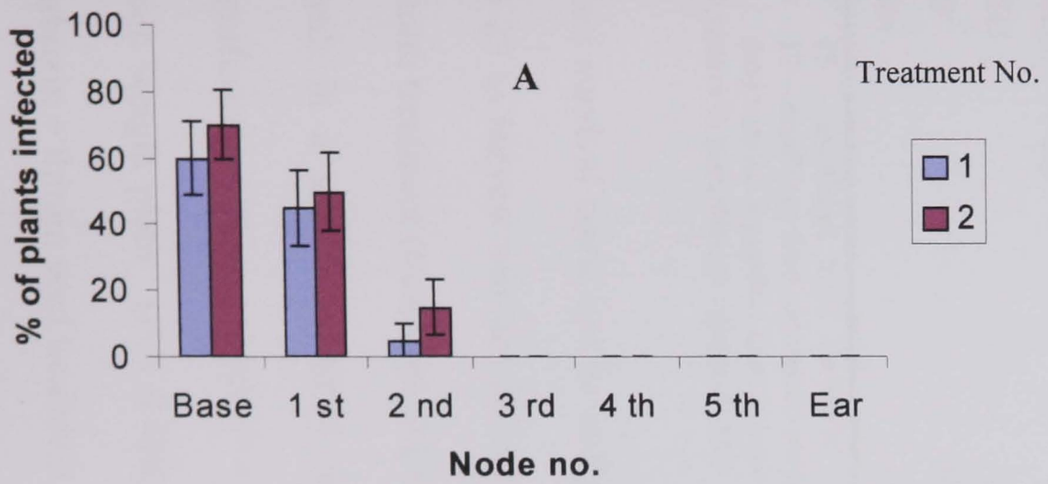


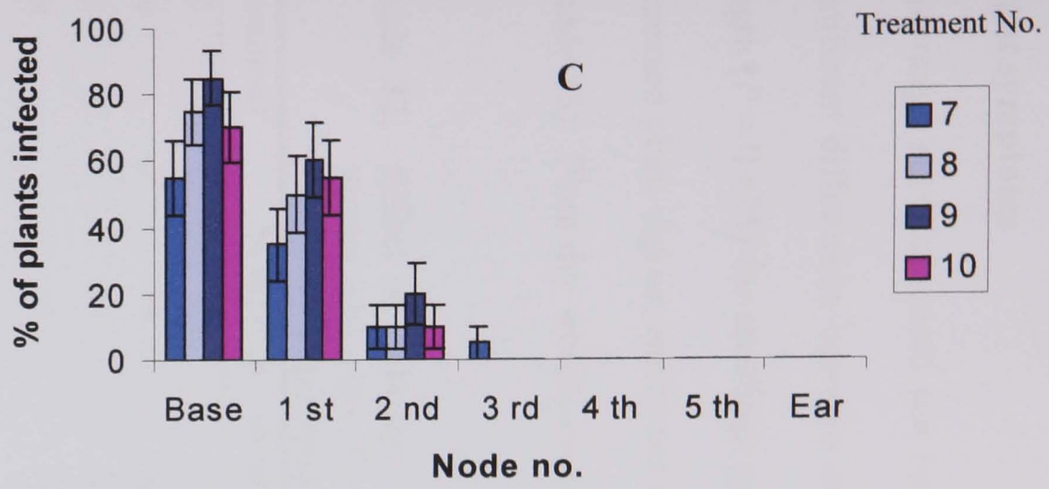
Figure 7. Effect of seedling blight severity, carboxin + thiram seed treatment and removal of seedling components on *Microdochium nivale* colonisation of winter wheat (cv. Cadenza) stems at GS 40-49 in glasshouse pot trials after seedling growth at 3 °C.

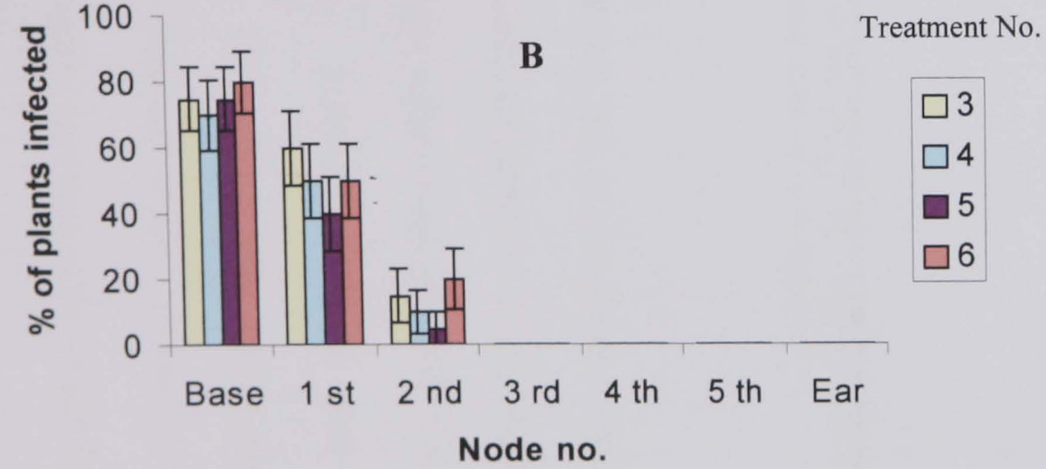
Bars represent SE.

A – seedlings from carboxin + thiram treated seeds; **B** – seedlings with disease score 1; **C** – seedlings with disease score 2; **D** – seedlings with disease score 3.

Treatment No.: **1** – seedlings from carboxin + thiram treated seeds; **2** – seeds removed from treatment 1; **3** – seedlings with disease score 1; **4** – seeds removed from treatment 3; **5** – coleoptiles removed from treatment 3; **6** – seeds and coleoptiles removed from treatment 3; **7** – seedlings with disease score 2; **8** – seeds removed from treatment 7; **9** – coleoptiles removed from treatment 7; **10** – seeds and coleoptiles removed from treatment 7; **11** – seedlings with disease score 3; **12** – seeds removed from treatment 11; **13** – coleoptiles removed from treatment 11; **14** – seeds and coleoptiles removed from treatment 11.







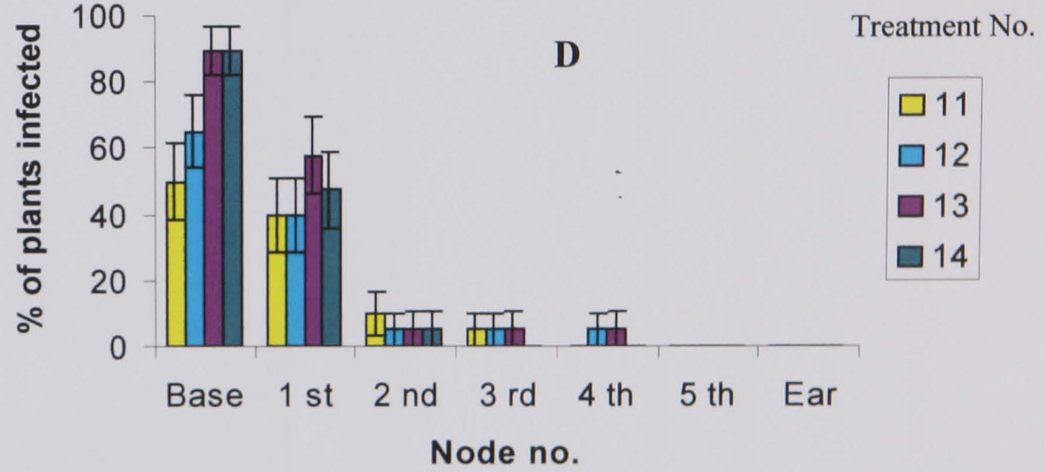


Figure 8. Effect of seedling blight severity, carboxin + thiram seed treatment and removal of seedling components on *Microdochium nivale* colonisation of winter wheat (cv. Cadenza) stems at harvest in glasshouse pot trials after seedling growth at 3 °C.

Bars represent SE.

A – seedlings from carboxin + thiram treated seeds; **B** – seedlings with disease score 1; **C** – seedlings with disease score 2; **D** – seedlings with disease score 3.

Treatment No.: **1** – seedlings from carboxin + thiram treated seeds; **2** – seeds removed from treatment 1; **3** – seedlings with disease score 1; **4** – seeds removed from treatment 3; **5** – coleoptiles removed from treatment 3; **6** – seeds and coleoptiles removed from treatment 3; **7** – seedlings with disease score 2; **8** – seeds removed from treatment 7; **9** – coleoptiles removed from treatment 7; **10** – seeds and coleoptiles removed from treatment 7; **11** – seedlings with disease score 3; **12** – seeds removed from treatment 11; **13** – coleoptiles removed from treatment 11; **14** – seeds and coleoptiles removed from treatment 11.

Effect of seed-borne *Microdochium nivale* infection and carboxin + thiram seed treatment on plant productivity and yield from seedlings not exhibiting seedling blight symptoms

Percentage survival could not be analysed due to a lack of normality. There were no significant differences between shoots plant^{-1} ($P = 0.557$), leaf area ($P = 0.427$) or stem length ($P = 0.425$) for seedlings grown at 22 °C. Carboxin + thiram seed treatment slightly increased plant vigour, measured by leaf area and dry weight, compared to untreated seeds (Table 33). Plant dry weight could not be analysed due to a lack of normality.

Table 32. Effect of carboxin + thiram seed treatment and seed removal on growth of winter wheat (cv. Cadenza) with seed-borne *Microdochium nivale* infection at GS 40-49 in pot trials after seedling growth at 22 °C.

treatment[1]	% survival	shoots plant^{-1}	leaf area (cm^2)		stem length (mm)		dry wt (g)	
15	97	4.38	246	5.5[2]	456	6.10[2]	1.99	(0.14)
16	99	4.43	233	5.4	467	6.14	2.11	(0.25)
17	99	4.28	218	5.3	443	6.06	1.79	(0.22)
18	95	4.54	221	5.3	466	6.12	1.92	(0.15)
LSD ($P < 0.05$)	-	NS	-	NS		NS	-	
SED	-	0.183	-	0.10		0.050	-	
DF	-	45	-	45		45	-	
%cv	-	13.1	-	6.4		2.6	-	

1 **15** – seedlings from carboxin + thiram treated seeds; **16** – seeds removed from treatment 15; **17** – seedlings from untreated seeds; **18** – seeds removed from treatment 17.
2 data natural logarithm transformed.
Numbers in parentheses represent SE.

Plant survival could not be analysed due to a lack of normality. Plant survival from GS 15-25 to harvest was not different for seedlings grown at 22 °C (Table 33). Carboxin + thiram treatment (treatment 15) significantly reduced ears plant^{-1} ($P < 0.05$) but this did not result in a significant yield reduction. Carboxin + thiram seed treatment did not significantly increase yield ($P = 0.229$), TGW ($P = 0.196$), grains plant^{-1} ($P = 0.512$) or grain weight plant^{-1} ($P = 0.420$) above untreated seeds. Of the yield components, only carboxin + thiram seed treatment significantly increased grain weight ear^{-1} ($P < 0.05$).

Table 33. Effect of carboxin + thiram seed treatment and seed removal on yield and its components of winter wheat (cv. Cadenza) with seed-borne *Microdochium nivale* infection in pot trials after seedling growth at 22 °C.

treatment[1]	% survival[2]	ears plant^{-1}		yield (g pot^{-1})		TGW	grains ear^{-1}	grain wt (g) ear^{-1}	grains plant^{-1}		grain wt (g) plant^{-1}	
15	100	3.03	1.73[3]	14.0	2.52[4]	40.0	29.2	1.16	89.9	4.42[4]	3.60	1.85[3]
16	99	3.26	1.79	16.2	2.68	41.4	30.7	1.27	101.2	4.52	4.17	1.99
17	100	3.61	1.89	15.0	2.63	41.7	27.2	1.08	93.8	4.47	3.79	1.91
18	100	3.61	1.89	14.7	2.50	37.3	28.1	1.06	101.5	4.50	3.90	1.89
LSD ($P < 0.05$)	-	-	0.118	-	NS	NS	2.84	0.152	-	NS	-	NS
SED	-	-	0.059	-	0.101	2.23	1.42	0.076	-	0.077	-	0.086
DF	-	-	51	-	51	51	51	51	-	51	-	51
%cv	-	-	10.2	-	12.4	17.6	15.6	21.0	-	5.5	-	14.1

1. **15** - seedlings from carboxin + thiram treated seeds; **16** - seeds removed from treatment 15; **17** - seedlings from untreated seeds; **18** - seeds removed from treatment 17.
2. from GS 15-25 to harvest.
3. data square root transformed.
4. data natural logarithm transformed.

Seed removal slightly increased grains plant^{-1} and grains ear^{-1} for plants from treated and untreated seeds. For carboxin + thiram treated seeds, seed removal also increased grain weight plant^{-1} and ear^{-1}.

Effect of seed-borne *Microdochium nivale* infection and carboxin + thiram seed treatment on foot rot incidence and stem colonisation on seedlings not exhibiting seedling blight symptoms

Disease incidence at GS 40-49 and harvest could not be analysed due to a lack of normality. At GS 40-49, *M. nivale* had not progressed beyond the first node of any plants from seedlings grown at 22 °C (Figure 9). Carboxin + thiram seed treatment reduced the incidence of *M. nivale* on stem-bases and first nodes. Seed removal had no effect on the incidence of *M. nivale*. *Microdochium nivale* incidence on the stem-base and first node declined from GS 40-49 to harvest on plants from carboxin + thiram treated seeds. However, *M. nivale* incidence on the first node was increased on plants from untreated seeds at harvest. *Microdochium nivale* was isolated from up to the second node on plants from untreated seeds and the first node for plants from treated seeds (Figure 10). *Microdochium nivale* disease incidence was greater on stem-bases and first nodes on plants from untreated seeds than plants from carboxin + thiram treated seeds at harvest.

DISCUSSION

This investigation has demonstrated for the first time that seed-borne *M. nivale* infection can cause foot rot, albeit in the absence of additional pathogens. Such observations contradict those of Rennie *et al.* (1983) who suggested that seed-borne *M. nivale* does not play a significant role in subsequent disease development. Turner *et al.* (2002) following field trials conducted at several sites within the UK between 1996-7 and 1998-9 also believed seed infection was not the predominant source of *M. nivale* inoculum for the

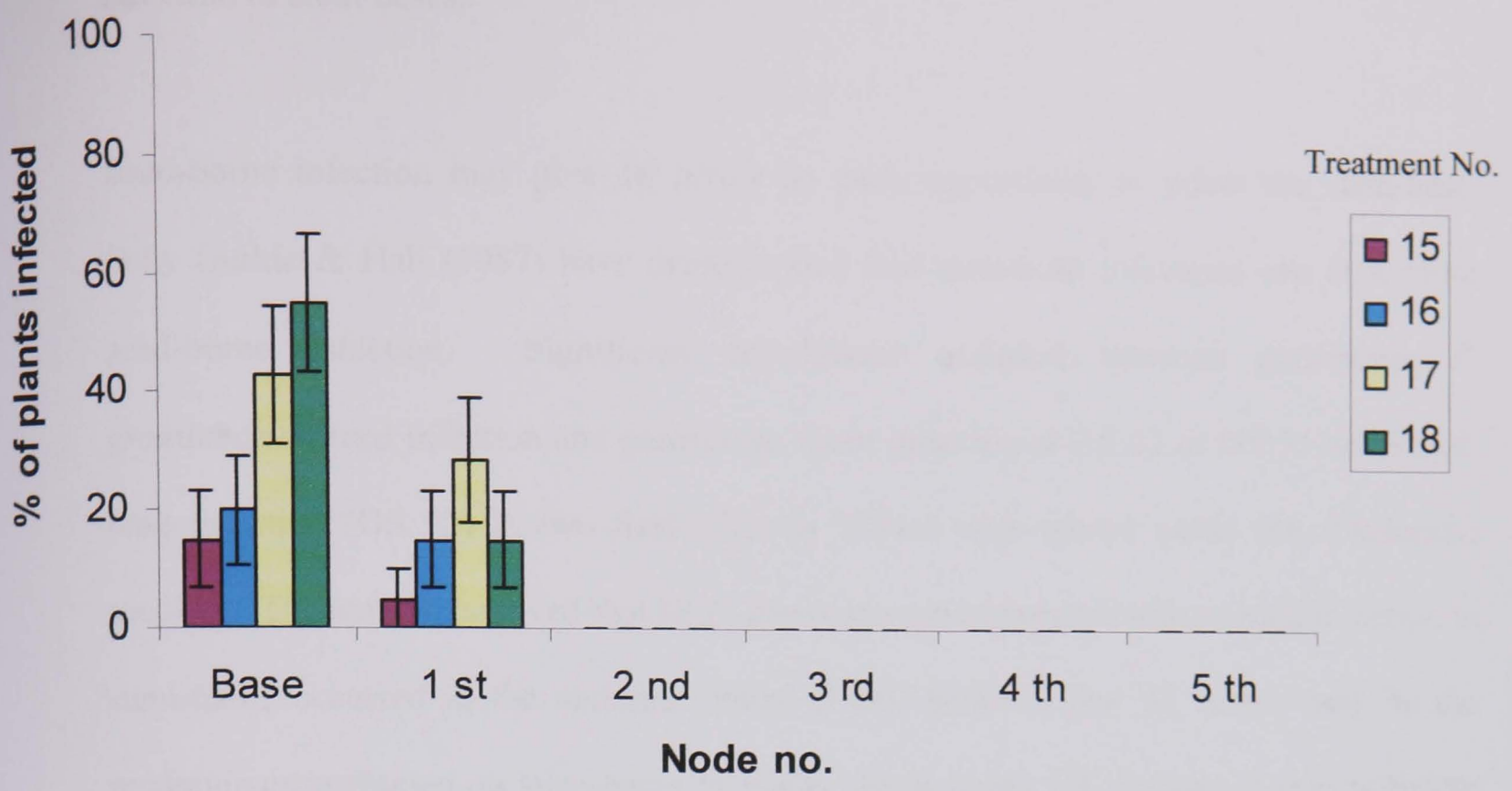


Figure 9. Effect of carboxin + thiram seed treatment and seed removal on stem colonisation of winter wheat (cv. Cadenza) from *Microdochium nivale* seed-borne infection at GS 40-49 in glasshouse pot trials after seedling growth at 22 °C.

Bars represent SE.

Treatment No.: **15** – seedlings from carboxin + thiram treated seeds; **16** – seeds removed from treatment 15; **17** – seedlings from untreated seeds; **18** – seeds removed from treatment 17.

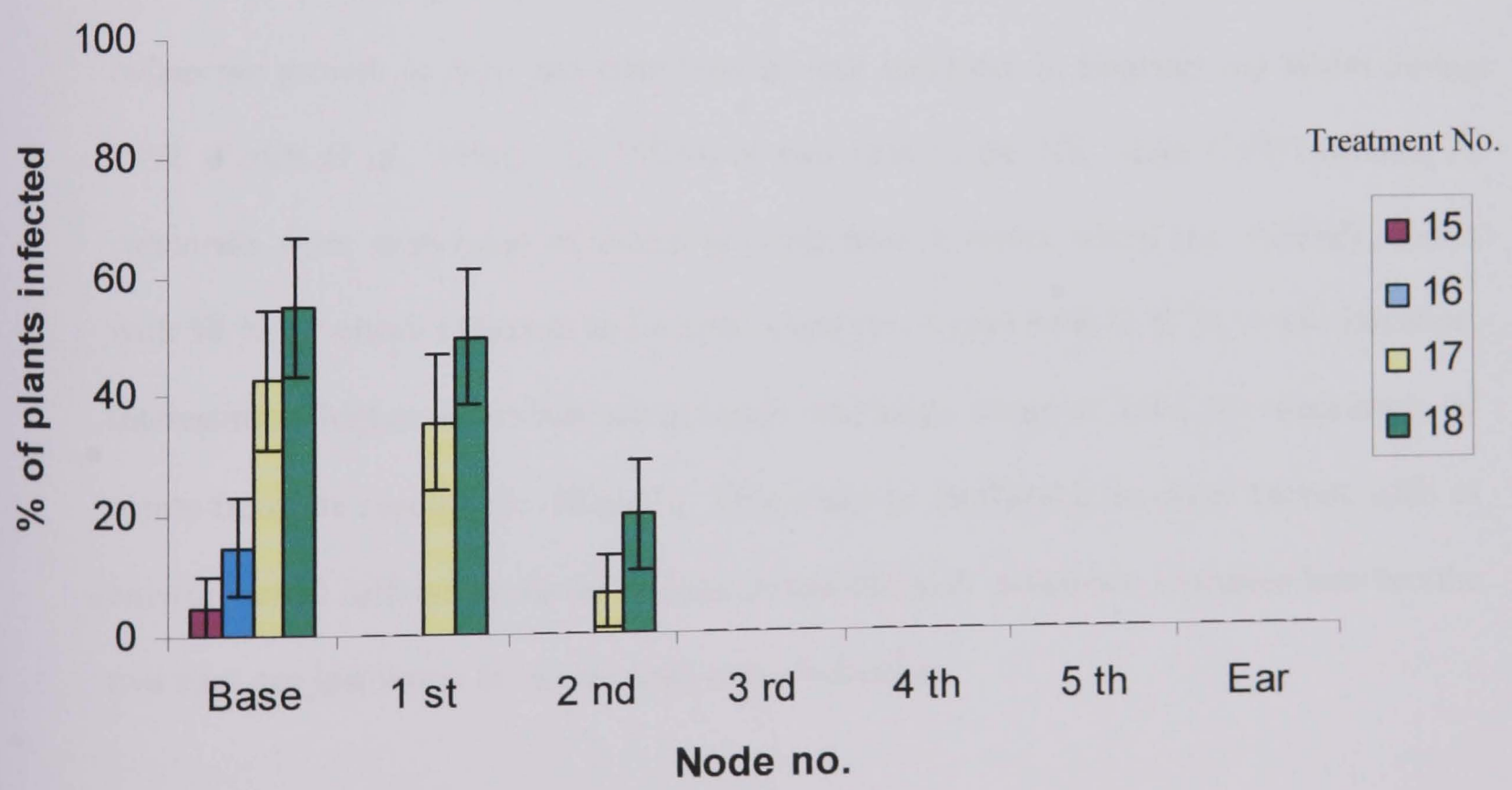


Figure 10. Effect of carboxin + thiram seed treatment and seed removal on stem colonisation of winter wheat (cv. Cadenza) from *Microdochium nivale* seed-borne infection at harvest in glasshouse pot trials after seedling growth at 22 °C.

Bars represent SE.

Treatment No.: **15** – seedlings from carboxin + thiram treated seeds; **16** – seeds removed from treatment 15; **17** – seedlings from untreated seeds; **18** – seeds removed from treatment 17.

infection of stem-bases.

Seed-borne infection may give *M. nivale* an early opportunity to infect the stem-base. Only Duthie & Hall (1987) have demonstrated that stem-base infections can arise from seed-borne infection. Significant correlations occurred between percentage *F. graminearum* seed infection and percentage shoot infection at GS 23 or GS 31 and shoot-base infection (GS 92) in two field trials in 1984-5 with winter wheat (cv. Frederick) seedlots. The authors believed that all *F. graminearum* transmission from seed infection to stem-bases occurred in the autumn. Parry (1990) believed that *M. nivale* may be the predominant pathogen on stem-bases of winter wheat in the UK because it infects before other *Fusarium* pathogens. Further evidence for *M. nivale* infecting the stem-base before other pathogens is provided by Pettitt *et al.* (1996) and Hare (1997). *Microdochium nivale* had a lower (1.5 °C) base temperature for growth *in vitro* than *F. culmorum* (5 °C). In addition, there were good correlations between accumulated thermal time, *M. nivale* and *F. culmorum* growth *in vitro* and stem-base disease incidence in England and Wales during 1992 (Pettitt *et al.*, 1996). In 1993-4 at two sites in the UK, Hare (1997) isolated *F. culmorum* from stem-bases of plants growing from a winter wheat (cv. Riband) seedlot with 38 % *M. nivale* infection and winter wheat (cv. Lynx) with 72 % *M. nivale* infection. Interestingly higher *F. culmorum* isolation incidence occurred from the stem-bases of plants from the seedlot (cv. Riband). This could be attributable to many factors, such as environmental differences or differences in soil-borne *F. culmorum* inoculum between the two sites not just lower *M. nivale* seed-borne infection.

In this investigation, seedling blight was not necessary for stem-base infections to occur, but the extent of stem colonisation was increased at GS 40-49 and at harvest on seedlings grown at 3 °C compared to 22 °C. This implies that *M. nivale* had already sufficiently colonised the seedlings from seed-borne infection to cause stem-base infections even at

22 °C. The mechanism by which *Fusarium* pathogens colonise wheat stems remains unclear. Snijders (1990) described systemic growth of *F. culmorum* from soil-borne spores reaching wheat internodes above node four, at GS 55-65 in pot trials. Soil-borne *F. culmorum*, *F. graminearum* and *M. nivale* colonised up to nodes four, two and two respectively, of winter wheat (cv. Cadenza) in conditions precluding splash dispersal (Clement & Parry, 1998). The mode of stem colonisation was not investigated here but at both sampling times, *M. nivale* was isolated from higher nodes of plants from heavily diseased seedlings. Greater *M. nivale* inoculum in coleoptile lesions and seeds might be expected to increase plant colonisation through splash dispersal or direct infection. Although analysis was not conducted, removal of seedling components did not appear to have a consistent effect on stem-base disease incidence or stem colonisation. Alternatively, severe seedling blight may delay plant growth, increasing the opportunity for further infection. However, it could be suggested that seedlings with disease severity three and possibly two, may not survive low winter temperatures.

The quantity of *M. nivale* present in stem-bases of wheat are reported to decline during the season (Parry, 1990). Locke *et al.* (1987) suggested this was due to late-formed shoot and stem-base sheath death, whilst Daamen *et al.* (1991) proposed gradually increasing temperatures were responsible, which may favour other stem-base pathogens, such as *F. culmorum* (Pettitt *et al.*, 1996). *Microdochium nivale* disease incidence typically increased between GS 40-49 and harvest in this investigation, probably because isolations were taken from the main shoots. That *M. nivale* could be isolated from the main shoot at harvest implies that death of plant parts is probably not responsible for reductions in *M. nivale* amounts in field grown winter wheat.

Heavily diseased seedlings produced shorter plants in this investigation. This is important since Jenkinson & Parry (1994) demonstrated that *F. culmorum* and *F. avenaceum* conidia

could be splash dispersed over relatively large distances from sporodochia on stem-bases. From observations that shorter wheat cultivars tended to have more *Fusarium* ear blight symptoms than taller cultivars in a trial in 1987, Mesterhazy (1995) speculated that taller wheat cultivars may hold ears further from sources of inoculum on stem-bases. When ears of the same cultivars were artificially inoculated under glasshouse conditions, no significant differences in disease severity occurred implying disease avoidance and not disease resistance was responsible for the field observations. Although not statistically analysed, removal of seed and seedling components from seedlings grown at 3 °C, reduced shoots plant^{-1} and plant dry weight and typically reduced leaf area and stem length. This implies there may be further undefined seed and coleoptile effects on plant growth.

Increased seedling blight severity reduced plant vigour, measured by shoots plant^{-1} and plant dry weight at GS 40-49. This is the first known evidence for seedling blight severity affecting subsequent winter wheat growth. Although a relationship between percentage seed infection and subsequent plant productivity has been shown. Duthie & Hall (1987) described a significant correlation between percentage seed-borne infection and shoots m^{-2} for plants of eight winter wheat seedlots with 0 to 43.5 % *F. graminearum* infection in 1984-5. However, in a trial with six winter wheat seedlots with 0 to 14.5 % infection, no such correlations occurred. The authors offered no reasons for this, but it is likely that the lower infection of seed in the second trial was responsible. During a field trial in 1991-2, Humphreys *et al.* (1995) observed a correlation between the extent of *M. nivale* seed infection (6 to 79 %), shoots plant^{-1} and ears m^{-2} for nine untreated wheat seedlots. In a similar field trial in 1991-2, similar trends occurred for six untreated oat cultivars with 5 to 61 % seed-borne *M. nivale* infection (Humphreys *et al.*, 1998). However, in these investigations, yield would be related to establishment and complicated by compensatory tillering and environmental effects. Further research is required to determine the effects of

disease pressure and source of inoculum on crop productivity, since the extent of seed-borne *M. nivale* infection affects subsequent plant growth.

Ears plant^{-1}, grain weight plant^{-1} and grains plant^{-1} declined slightly but not significantly, with increased seedling blight severity. However, only plants from heavily diseased seedlings (treatments 11-14) had consistently reduced yield and TGW. Removal of seed and seedling components had no consistent effect on yield and its components. *Fusarium avenaceum*, *F. culmorum*, *F. graminearum* and *M. nivale* inoculation of glasshouse grown winter wheat (cv. Avalon) stem-bases at GS 21 has previously been shown to reduce yield, TGW and grains ear^{-1} (Hutcheon & Jordan, 1992). However, it was not clear what caused the reductions in Hutcheon & Jordan's trial. Additionally, amounts of inoculum were probably much higher than those present in this study and were specifically targeted at the stem-base.

Seed treatments through reductions in seedling blight incidence may lower the amount of inoculum present, reducing subsequent plant colonisation. Carboxin + thiram seed treatment reduced stem-base disease incidence and stem colonisation compared to untreated seeds for seedlings grown at 3 and 22 °C. Disease incidence and stem colonisation on plants from treated seeds did not increase between GS 40-49 and harvest. Increased stem-base and first node disease incidence and second node infection occurred on plants from treated seedlings grown at 3 °C compared to 22 °C. Reasons for this are not obvious but may be due to increased disease pressure at lower temperatures. Removal of treated seeds slightly increased disease incidence and stem colonisation at both temperatures. This may be attributed to systemic control of disease by carboxin + thiram that had escaped initial control. Maneb, carboxin and triadimenol seed treatments reduced winter wheat stem-base infection from soil-borne *Fusarium* inoculum compared to untreated seeds in field trials during 1981-2 and 1982-3 (Celetti & Hall, 1987). In a pot

trial, eight seed treatments reduced the number of shoots infected at GS 31 and disease severity on the top three internodes compared to an untreated control (Hutcheon & Jordan, 1992). However, only tebuconazole significantly reduced basal stem severity, measured by mean lesion area.

Seed treatments might be expected to improve plant productivity through reduced stem colonisation. Plant survival from carboxin + thiram treated seeds grown at 3 °C was very high and treated seeds produced taller plants with greater leaf area and plant dry weight. Escape from seedling blight and continued disease pressure may increase plant vigour from treated seeds. However, yield from carboxin + thiram treated seeds was only increased above heavily diseased seedlings (disease scores two and three). This may suggest that slight seedling blight does not adversely affect subsequent plant growth and yield, whilst severe seedling blight from heavily infected untreated seeds allows continued *M. nivale* infection of plants. Rawlinson & Colhoun (1970) attributed increased oat vigour and yield from organomercury treated pathogen-free seeds to mesocotyl protection from soil-borne fungi.

Removal of treated seeds in this trial appeared to reduce plant vigour, but did not adversely affect yield. For seedlings grown at 22 °C, carboxin + thiram treatment only increased leaf area and plant dry weight but did not consistently increase yield above untreated seeds. This may imply that seed-borne inoculum is insufficient alone to adversely affect plant growth under conditions not conducive for seedling blight. Triadimenol + bitertanol + fuberidazole, tebuconazole + thiram and bitertanol + fuberidazole significantly reduced diseased ear area, but no treatments significantly increased yield of winter wheat growing in pots containing soil-borne *Fusarium* inoculum (Hutcheon & Jordan, 1992). Additionally, triadimenol, diniconazole and tebuconazole significantly reduced the incidence of maize head smut initiated from soil-borne inoculum in pot trials at three sites

in 1991 and 1992 in Nepal (Pradhanang & Ghimire, 1996). Unfortunately yield data were not presented.

Further beneficial effects from seed treatments to plants under exposure to pathogens similar in biology to *Fusarium* spp. have been reported. El-Tayeb *et al.* (1987) described five treatments increasing leaf number, leaf area, plant height, root length, shoot number, plant fresh weight and dry weight over untreated seeds for two wheat cultivars, three months after sowing seeds surface-inoculated with *F. roseum* and *Alternaria alternata* spores. The seed treatments also generally increased head length, spikes plant^{-1}, kernels spike^{-1}, spikelets plant^{-1}, kernels spikelet^{-1}, TGW and yield. Across two sites in 1993, plants from metalaxyl + carboxin + furathiocarb treated seeds had significantly increased plant height, root structure, yield, seeds ear^{-1} and seed weight ear^{-1} compared to plants from untreated wheat seeds (cv. Siete Cerros) growing in soil containing *F. equiseti* and *Exserohilum rostratum* inoculum (Marley & Adeoti, 1995). In all instances, seed treatments were probably improving plant productivity by reducing disease challenge.

CHAPTER 8

Effects of fluctuating temperatures and soil water contents, and fungicide seed treatments on seedling emergence, *Microdochium nivale* seedling blight severity, foot rot disease incidence and subsequent winter wheat growth and yield

INTRODUCTION

As discussed previously, control of *Fusarium* seedling blight through fungicide seed treatments can significantly increase establishment and reduce seedling blight severity. *Microdochium nivale* causes most severe seedling blight in cold dry soils (Millar & Colhoun, 1969b). Hare *et al.* (1995) described reduced temperatures (6 and 8 °C) only reducing the efficacy of thiabendazole and flutriafol seed treatments, measured by final emergence from a *M. nivale* infected winter wheat seedlot. However, no investigations into the effects of fluctuating soil temperatures and soil water regimes on *M. nivale* seedling blight, seed treatment performance and their interactions have been conducted.

Only Paveley & Davies (1994) have highlighted inconsistent seed treatment performance against soil-borne seedling blight diseases at different sites in the UK. Final emergence from a pathogen-free winter wheat (cv. Riband) seedlot treated with six fungicide seed treatments was variable across nine sites between 1992-4. A similar trend occurred across six sites for a winter barley (cv. Puffin) seedlot treated with six fungicide seed treatments. However, due to the experimental design, differences observed between sites could be due to widely differing soil types and seed bed conditions. The aims of the work in this chapter were to: (i) determine the effect of seed-borne *M. nivale* on winter wheat emergence and seedling blight severity under field conditions. (ii) attempt to characterise critical phases of seedling growth and the effects of fluctuating temperatures and soil water contents on the winter wheat-*M. nivale* interaction, which affects seedling emergence. (iii) investigate the effects of temperature and soil water content on field performance of seed treatments. (iv) evaluate the effectiveness of seed treatments in controlling seedling blight and foot rot. (v) investigate the effect of seed-borne *M. nivale* and seed treatments on subsequent crop growth and yield through 18 field trials over three years.

MATERIALS & METHODS

Seed treatments used in the following experiments are given in Table 34. Treatments were applied at the manufacturers' recommended use rates (Table 5) or the seed left untreated. Seedlots were drilled at 400 seeds m^{-2} using a small plot drill.

Table 34. Details of fungicides used as seed treatments in three years of field trials.

active ingredient(s)	year		
bitertanol + fuberidazole	1999-2000;	2000-2001;	2001-2002
carboxin + thiram	1999-2000;	2000-2001;	2001-2002
fludioxonil	1999-2000		
fluquinconazole + prochloraz	2000-2001;	2001-2002	
guazatine	1999-2000		

The efficacy of fungicide seed treatments under different field conditions

An experiment was designed to test the hypothesis that field seedbed conditions, specifically soil temperature have no effect on final emergence and establishment from a winter wheat seedlot treated with fungicides or left untreated. Seedlot 9 (cv. Riband; 56 % infection) (Table 2: page 33) was drilled at eight sites throughout the UK in 1999-2000. The sites were Caythorpe in Lincolnshire, Edinburgh in Lothian, Harper Adams University College (Edgmond) in Shropshire (three trials), High Mowthorpe in North Yorkshire, Morley in Norfolk and Rosemaund in Herefordshire (for details of the trial sites, see Table 35). Trials were drilled in 12 x 1.75 m plots (6 x 1.75 m at Harper Adams University College) according to randomised block designs with four replicates for each treatment.

Final emergence (GS 10-12) and establishment (GS 15-25) were recorded for five 0.5 m row lengths in each plot. Plant counts were expressed as number of plants m^{-2}. Soil temperature during emergence was recorded and the median daily soil temperature calculated (Chapter 3).

For data at the same site, ANOVAs were conducted with final emergence and establishment as variables and seed treatments as factors. For combined data sets across all sites, data were factorially ANOVA analysed with seed treatments and sites as factors. Linear regression analyses were conducted to determine possible interactions between seed treatments and daily median soil temperatures for final emergence across all sites. Regression analysis was also conducted between final emergence and establishment across all sites.

Table 35. Information for the field trial sites in 1999-2000.

site	drilling date	soil type	sowing depth (cm)	pH	previous crop	cultivations
Caythorpe	10/11/99	loam	2.5 – 3.5	7.7	spring barley	plough, power harrow
Edinburgh	26/11/99	loam	2.0 – 3.0	5.8	spring barley	plough
Harper Adams (1)	18/10/99	sandy loam	2.5 – 4.0	6.4	potatoes	plough, power harrow
Harper Adams (2)	23/11/99	sandy loam	2.5 – 4.0	6.4	potatoes	plough, power harrow
Harper Adams (3)	21/01/00	sandy loam	2.5 – 4.0	6.4	potatoes	plough, power harrow
High Mowthorpe	02/11/99	silty clay loam	2.2	8.0	winter oilseed rape	plough, power harrow, roll
Morley	12/11/99	sandy loam	2.5 – 4.5	8.1	sugar beet	plough, press, power harrow
Rosemaund	10/11/99	silty clay loam	3.0 – 4.0	7.3	potatoes	plough, power harrow

Fungicide seed treatment efficacy and winter wheat performance in field trials

Field trials were conducted over two years to test the hypothesis that (i) critical phases of soil water and temperature have no effect on seedling emergence from treated and untreated seedlots, (ii) rate of emergence is not correlated with final emergence and seedling blight severity for treated and untreated seedlots, (iii) seed-borne *M. nivale* infection has no adverse effects on subsequent crop growth and yield, (iv) seed treatment control of seedling blight has no effect on subsequent crop growth and yield. In each year, seedlots of the same cultivar with different amounts of *M. nivale* infection were used to enable investigations into the effects of the extent of *M. nivale* infection on seed treatment efficacy and winter wheat performance. Seedlots 4 (cv. Equinox; 8 % infection) and 6

(cv. Equinox; 88 % infection) (Table 2: page 33) were used in 2000-1. Trials were drilled on; 25/10/2000 (trial 1), 10/01/2001 (trial 2), 25/01/2001 (trial 3), 09/02/2001 (trial 4) and 21/02/2001 (trial 5) into a sandy loam soil after ploughing and power harrow cultivations. Seedlots 1 (cv. Cadenza; 0 % infection) and 2 (cv. Cadenza; 29 % infection) (Table 2: page 33) were used in 2001-2. Trials were drilled on ,13/10/01 (trial 1), 03/11/01 (trial 2), 09/11/01 (trial 3) and 13/12/01 (trial 4) into a sandy loam soil after ploughing and power harrow cultivations. Trials were drilled in 6 m x 1.75 m plots according to randomised block designs with five replicates for each treatment.

Final emergence (GS 10-12) and establishment (GS 15-25) were recorded for five 0.5 m lengths of row per plot. During emergence, seedling counts were made every two days and rate of emergence calculated (Equation 2). Soil temperature was recorded hourly and the median daily soil temperature calculated (Chapter 3). Water content in the top 5 cm of soil was determined every two days from eight cores in discard plots (Chapter 3). Soil temperature and soil water content were used for regression analysis.

Seedling blight disease assessments were made at GS 10-12 and GS 15-25 (Table 4). Foot rot incidence was determined at GS 39 and GS 75 (Equation 4). At all sampling times, 25 plants were taken from the edge of each plot in a W-pattern. Shoot counts were made at GS 39 and ear counts at GS 75 from five 0.5 m lengths of rows per plot. Final emergence, establishment, shoot and ear counts were converted to numbers m^{-2}. Yield and TGW were determined at harvest and adjusted to 15 % moisture.

For data at the same drilling date, factorial ANOVAs were conducted with rate of emergence, final emergence, establishment, shoots m^{-2}, ears m^{-2}, yield, TGW and where appropriate, foot rot disease incidence as variables and seed treatment and seedlot as factors. For combined data sets across all drilling dates, data were analysed as a factorial

ANOVA with seed treatment, seedlot and drilling date as factors. Where required, data were transformed to ensure normally distributed data. Where data could not be transformed to normality, standard errors were calculated. Linear regressions were conducted to determine possible interactions between seed treatment, *M. nivale* infection, soil temperature and soil water content affecting final emergence. A possible relationship between rate of emergence, final emergence, establishment, shoots m^{-2}, ears m^{-2} and yield across all drilling times was also investigated. Where appropriate, linear regressions between foot rot disease incidence at GS 39 and GS 75 were also conducted.

RESULTS

The efficacy of fungicide seed treatments under different field conditions

Seed treatments significantly increased final emergence above untreated seeds across all sites ($P < 0.05$). Trial location also significantly influenced final emergence ($P < 0.05$). The trial location x seed treatment interaction was not significant ($P = 0.090$). Seed treatment performance was not consistent across all sites, although carboxin + thiram treatment always produced the first or second highest final emergence (Table 36). Only in the first and second drillings at Harper Adams University College were significant differences observed between seed treatments for final emergence.

Seed treatments significantly increased establishment above untreated seeds across all sites ($P < 0.05$). Trial location also significantly influenced establishment ($P < 0.05$) but the seed treatment x site interaction was not significant ($P = 0.169$). Highest establishment came from carboxin + thiram treated seeds at all sites (Table 37). Across all sites except Edinburgh, Harper Adams University College second drilling and High Mowthorpe, carboxin + thiram significantly increased establishment above at least one other seed treatment ($P < 0.05$). Linear regression showed a good relationship between final

emergence and establishment ($y = 0.7195x + 40$; $R^2 = 0.719$; $P < 0.001$). Good correlations were obtained for median temperature on day five after drilling and final emergence for all untreated and treated seeds except carboxin + thiram and fludioxonil seed treatments (Figure 11).

Table 36. Effect of seed treatments and site on final emergence (m^{-2}) from a winter wheat seedlot (cv. Riband) with 56 % *Microdochium nivale* infection in eight trials across the UK in 1999-2000.

	plant emergence (m^{-2})								
	site								
treatment	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**COMB**
untreated	213	195	246	211	156	190	159	159	191
bitertanol + fuberidazole	263	247	279	276	195	247	213	251	246
carboxin + thiram	289	284	306	304	287	241	253	260	278
fludioxonil	262	243	263	269	217	229	252	241	247
guazatine	267	253	306	265	247	237	229	263	258
LSD ($P < 0.05$)	32.2	49.3	32.8	30.1	31.1	18.2	61.4	41.3	35.6
SED	14.8	22.6	15.1	13.8	14.3	8.4	28.2	18.9	17.9
DF	15	15	15	15	15	15	15	15	96
%cv	8.1	13.1	7.6	7.4	9.1	5.2	18.0	11.4	10.4

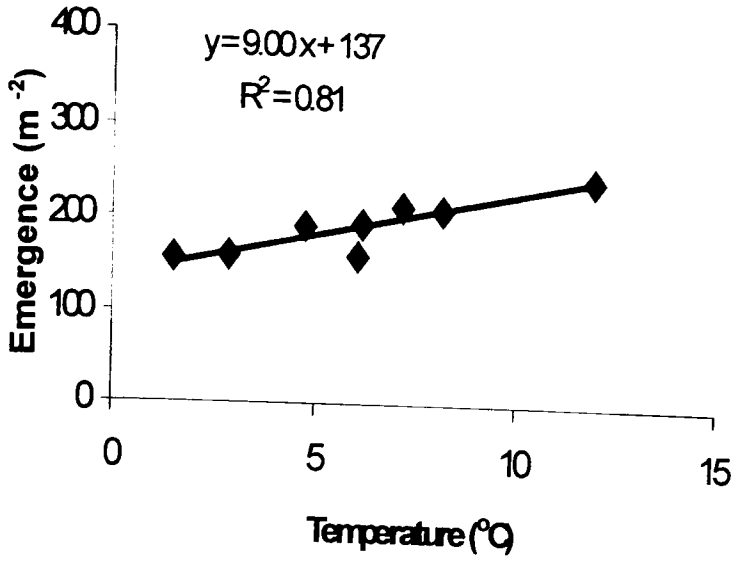
1 - Caythorpe; **2** - Edinburgh; **3** - Harper Adams 1; **4** - Harper Adams 2; **5** - Harper Adams 3; **6** - High Mowthorpe; **7** - Morley; **8** - Rosemaund.
Seedlot **9**; Table 2; page 33.

Table 37. Effect of seed treatments and site on establishment (m^{-2}) for a winter wheat seedlot (cv. Riband) with 56 % *Microdochium nivale* infection in eight trials across the UK in 1999-2000.

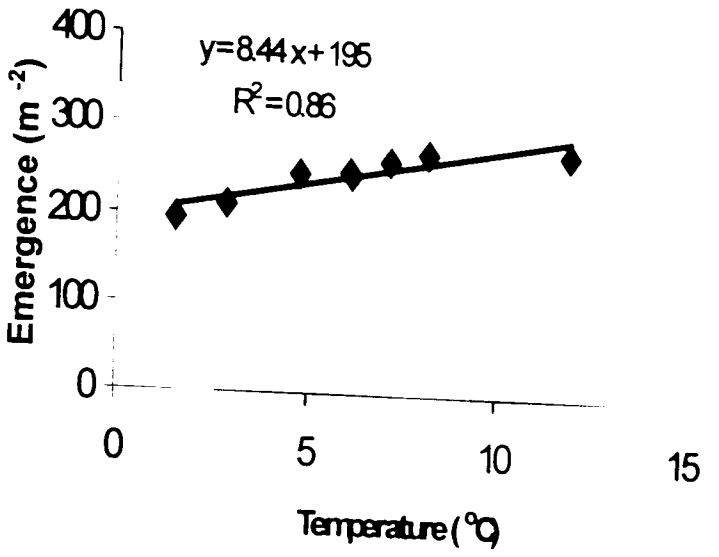
	plant establishment (m^{-2})								
	site								
treatment	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**COMB**
untreated	185	181	220	186	139	161	155	165	174
bitertanol + fuberidazole	210	237	248	234	167	208	205	223	217
carboxin + thiram	250	269	271	237	215	213	260	247	245
fludioxonil	213	235	234	211	179	196	251	213	217
guazatine	225	238	271	229	193	202	225	224	226
LSD ($P < 0.05$)	31.1	45.0	29.1	32.7	26.1	20.5	44.0	18.1	29.3
SED	14.3	20.7	13.3	15.0	12.0	9.4	20.2	8.3	13.8
DF	15	15	15	15	15	15	15	15	96
%cv	9.3	12.6	7.6	9.7	9.5	6.8	13.0	5.5	9.7

1 - Caythorpe; **2** - Edinburgh; **3** - Harper Adams 1; **4** - Harper Adams 2; **5** - Harper Adams 3; **6** - High Mowthorpe; **7** - Morley; **8** - Rosemaund.
Seedlot **9**; Table 2; page 33.

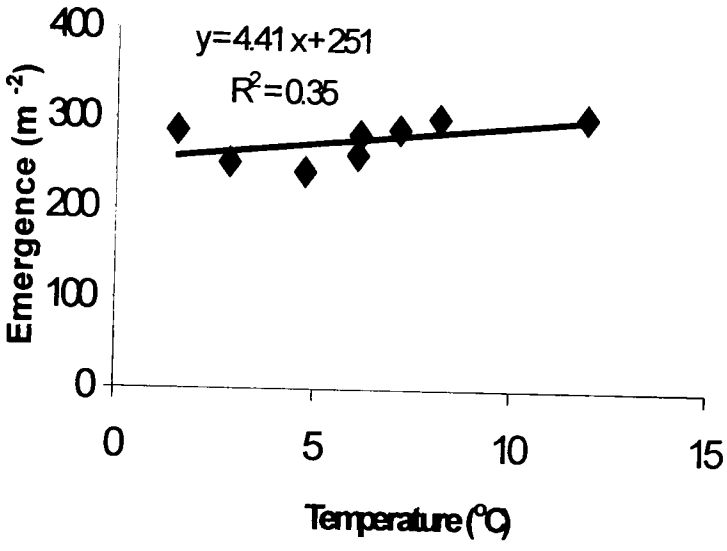
untreated seeds



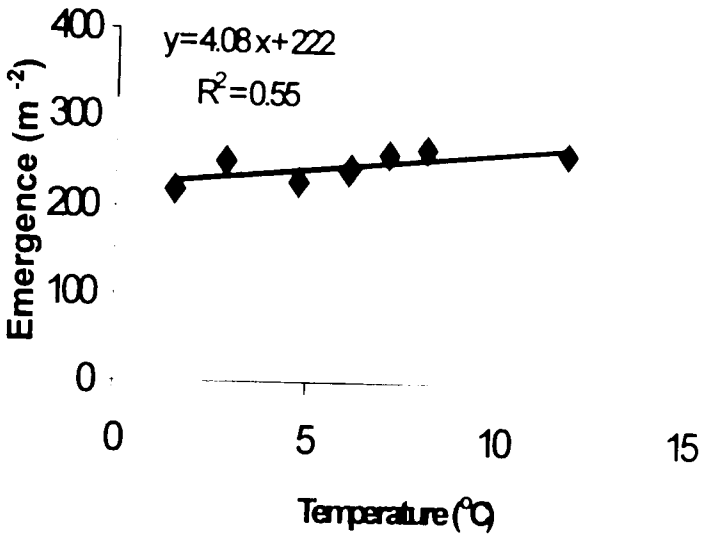
bitertanol + fuberidazole treated seeds



carboxin + thiram treated seeds



fludioxonil treated seeds



guazatine treated seeds

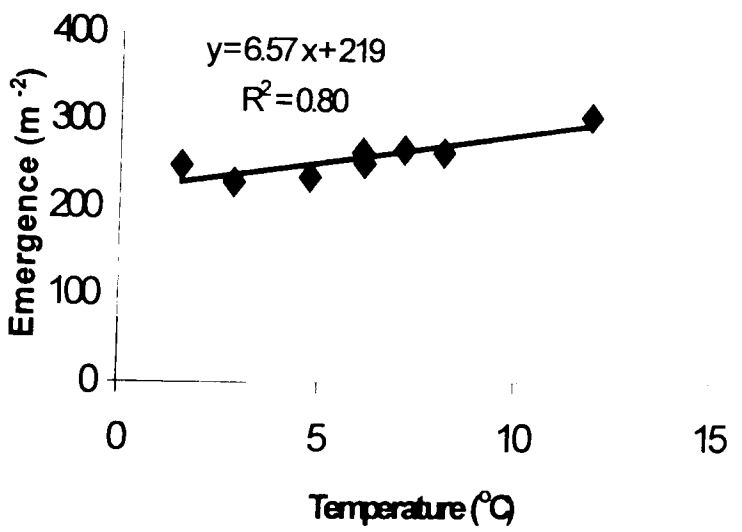


Figure 11. Effect of median temperature on day five after drilling on final emergence from a winter wheat seedlot (cv. Riband: seedlot 9) with 56 % *Microdochium nivale* infection with and without fungicide seed treatments at eight trial sites across the UK in 1999-2000.

Fungicide seed treatment efficacy and winter wheat performance in field trials during 2000-2001

Seedling emergence

Rate of emergence was not significantly different from each seedlot (P = 0.331). Fluquinconazole + prochloraz significantly reduced rate of seedling emergence from both seedlots across all drilling dates ($P < 0.05$). Time of drilling significantly affected rate of emergence (Table 38). Seedlings from trial 1 emerged significantly faster than all other trials and seedlings from trial 2 emerged the slowest ($P < 0.05$). The seedlot x seed treatment interaction was significant ($P < 0.001$). Rate of emergence from carboxin + thiram (0.0304 day^{-1}) and bitertanol + fuberidazole (0.0306 day^{-1}) treated seeds of seedlot 4 was significantly slower ($P < 0.05$) than seedlot 6 (carboxin + thiram treated seeds 0.0314 day^{-1}; 0.0296 day^{-1}). The opposite trend occurred for untreated seeds (seedlot 4, 0.0309 day^{-1}; seedlot 6, 0.0317 day^{-1}). The seedlot x seed treatment x drilling date interaction was significant ($P < 0.001$) but for both seedlots bitertanol + fuberidazole treated seeds emerged the fastest.

Final emergence was significantly greater ($P < 0.05$) in trial 1 (230 m^{-2}) than in trials 2 (138 m^{-2}), 3 (138 m^{-2}), 4 (140 m^{-2}) and 5 (141 m^{-2}). Final emergence was significantly ($P < 0.05$) higher from seedlot 6 (88 % infection; 162 m^{-2}) than seedlot 4 (8 % infection; 134 m^{-2}). Final emergence from carboxin + thiram treated seeds (198 m^{-2}) was significantly above all other treatments ($P < 0.05$). Final emergence from untreated seeds (92 m^{-2}) was significantly (P <0.05) below emergence from bitertanol + fuberidazole (173 m^{-2}) and fluquinconazole + prochloraz (168 m^{-2}) treated seeds (Table 39). The seedlot x seed treatment interaction was significant ($P < 0.001$). Final emergence from seedlot 6 was significantly greater ($P < 0.05$) than seedlot 4 for carboxin + thiram (seedlot 4, 163 m^{-2}; seedlot 6, 234 m^{-2}), bitertanol + fuberidazole (seedlot 4, 134 m^{-2}; seedlot 6, 211 m^{-2}) and fluquinconazole + prochloraz (seedlot 4, 126 m^{-2}; seedlot 6 , 210 m^{-2}) treated

Table 38. Effect of seed treatments and drilling date on rate of emergence (day^{-1}) for two winter wheat seedlots (cv. Equinox) infected with *Microdochium nivale* in field trials at Harper Adams University College in 2000-2001.

	rate of emergence (day^{-1})						
	trial*						
treatment	1	2	3	4	5	combined	
	8 % *M. nivale* infection (seedlot 4)						
untreated	0.0469	0.0221	0.0262	0.0279	0.0299	0.0306	-3.520[1]
bitertanol + fuberidazole	0.0476	0.0222	0.0268	0.0283	0.0296	0.0309	-3.511
carboxin + thiram	0.0462	0.0218	0.0262	0.0275	0.0303	0.0304	-3.526
fluquinconazole + prochloraz	0.0406	0.0210	0.0245	0.0272	0.0284	0.0284	-3.588
	88 % *M. nivale* infection (seedlot 6)						
untreated	0.0430	0.0218	0.0256	0.0285	0.0289	0.0296	-3.547
bitertanol + fuberidazole	0.0501	0.0205	0.0269	0.0282	0.0308	0.0317	-3.492
carboxin + thiram	0.0494	0.0224	0.0261	0.0286	0.0305	0.0314	-3.499
fluquinconazole + prochloraz	0.0415	0.0223	0.0244	0.0267	0.0286	0.0283	-3.591
LSD ($P < 0.05$)	0.00200	0.00050	0.00091	0.00090	0.00094	-	0.0325
SED	0.00097	0.00025	0.00045	0.00044	0.00046	-	0.0164
DF	28	28	28	28	28	-	140
%cv	3.4	1.8	2.7	2.5	2.4	-	0.7

* **trial 1** drilled 25/10/2000, **trial 2** drilled 10/01/2001, **trial 3** drilled 25/01/2001, **trial 4** drilled 09/02/2001, **trial 5** drilled 21/02/2001.

1 data natural logarithm transformed.

Seedlot **4** (8 % *Microdochium nivale* infection), seedlot **6** (88 % *Microdochium nivale* infection), see Table 2: page 33.

seeds. The opposite trend occurred for untreated seeds (seedlot 4, 112 m^{-2}; seedlot 6, 72 m^{-2}).

Establishment was significantly greater ($P < 0.05$) in trial 1 (153 m^{-2}) than in trials 2 (121 m^{-2}), 3 (120 m^{-2}), 4 (134 m^{-2}) and 5 (130 m^{-2}). Establishment was significantly ($P < 0.05$) higher from seedlot 6 (148 m^{-2}) than seedlot 4 (115 m^{-2}) across all drilling dates. Establishment from carboxin + thiram treated seeds (162 m^{-2}) was significantly ($P < 0.05$) above all treatments. Final emergence from untreated seeds (77 m^{-2}) was significantly ($P < 0.05$) below emergence from bitertanol + fuberidazole (143 m^{-2}) and fluquinconazole + prochloraz (144 m^{-2}) treated seeds (Table 39). The seedlot x seed treatment interaction was significant ($P < 0.001$). Establishment from seedlot 6 significantly greater ($P < 0.05$)

Table 39. Effect of seed treatments and drilling date on final emergence (m^{-2}) at GS 10-12 and establishment (m^{-2}) at GS 15-25 from two winter wheat seedlots (cv. Equinox) infected with *Microdochium nivale* in field trials at Harper Adams University College in 2000-2001.

	final emergence (m^{-2})						establishment (m^{-2})					
	trial*						trial*					
treatment	**1**	**2**	**3**	**4**	**5**	**COMB**	**1**	**2**	**3**	**4**	**5**	**COMB**
						8 % *M. nivale* infection (seedlot 4)						
untreated	165	97	96	98	102	112	115	89	88	94	97	97
bitertanol + fuberidazole	211	121	109	115	115	134	143	109	88	111	103	111
carboxin + thiram	213	151	142	145	162	163	165	138	125	141	150	144
fluquinconazole + prochloraz	212	98	103	107	109	126	157	86	96	105	105	110
						88 % *M. nivale* infection (seedlot 6)						
untreated	139	45	54	64	56	72	87	36	50	61	56	58
bitertanol + fuberidazole	289	177	202	197	191	211	197	150	169	188	169	175
carboxin + thiram	307	219	222	215	206	234	150	183	188	193	184	179
fluquinconazole + prochloraz	304	200	179	180	187	210	208	179	153	177	177	179
LSD ($P < 0.05$)	35.9	23.5	25.4	22.0	33.0	27.5	29.8	23.7	20.8	22.0	27.5	24.0
SED	17.5	11.5	12.4	10.8	16.1	13.9	14.5	11.6	10.1	10.7	13.4	12.2
DF	28	28	28	28	28	140	28	28	28	28	28	140
%cv	12.0	13.1	14.2	12.1	18.0	13.9	15.0	15.1	13.4	12.7	16.3	14.7

* **trial 1** drilled 25/10/2000, **trial 2** drilled 10/01/2001, **trial 3** drilled 25/01/2001, **trial 4** drilled 09/02/2001, **trial 5** drilled 21/02/2001.

For more information on the seedlots, refer to Table 2; page 33.

than seedlot 4 for carboxin + thiram (seedlot 4, 144 m^{-2}; seedlot 6, 179 m^{-2}), bitertanol – fuberidazole (seedlot 4, 111 m^{-2}; seedlot 6, 175 m^{-2}) and fluquinconazole + prochloraz (seedlot 4, 110 m^{-2}; seedlot 6 , 179 m^{-2}) treated seeds. The opposite trend occurred for untreated seeds (seedlot 4, 97 m^{-2}; seedlot 6, 58 m^{-2}). Across all drilling dates, establishment from seedlot 6 was significantly above seedlot 4 ($P < 0.05$). Establishment from untreated seeds was significantly below ($P < 0.05$) establishment from treated seeds. Establishment from carboxin + thiram treated seeds was significantly above establishment from bitertanol + fuberidazole and fluquinconazole + prochloraz treated seeds, except for the first drilling date. The seedlot x seed treatment x drilling date interaction was significant ($P = 0.007$) but across all drilling dates establishment for untreated seeds was significantly below treated seeds for both seedlots.

Rate of emergence was significantly correlated ($R^2 = 0.605$; $P < 0.001$) with final emergence for each seed treatment. Final emergence and establishment were significantly correlated ($R^2 = 0.842$; $P < 0.001$) for each seed treatment. Regression analysis revealed that median temperature on day four and five after drilling was most closely correlated to final emergence for both seedlots (Figure 12). Multiple linear regression analysis of soil water content and temperature revealed that the period from drilling to thirty days post drilling was generally most related to final emergence of seedlots 4 and 6 (Table 40).

Subsequent plant productivity

Drilling date had a significant effect on shoots m^{-2} ($P < 0.001$). Plants from trial 1 produced the most shoots (480 m^{-2}). Plants from seedlot 6 (416 m^{-2}) produced significantly ($P < 0.05$) more shoots m^{-2} than seedlot 4 (371 m^{-2}). Seed treatment significantly increased ($P < 0.001$) shoots m^{-2}. Plants from carboxin + thiram treated seeds (437 m^{-2}) had significantly more ($P < 0.05$) shoots than bitertanol + fuberidazole treated

Table 40. Multiple regression analysis for the effect of median daily temperature and soil water content between drilling and thirty days post drilling on final emergence from two winter wheat seedlots (cv. Equinox) infected with *Microdochium nivale* during field trials at Harper Adams University College in 2000-2001.

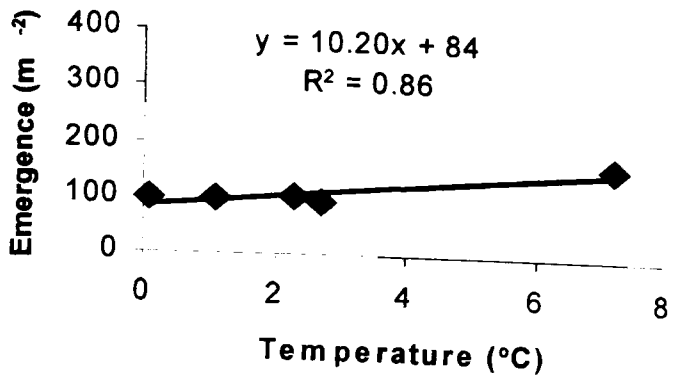
seedlot	seed treatment	equation	R^2	*P*
4	untreated	$y = -38.8 + 12.81x_i + 4.20x_{ii}$	0.923	0.038
4	bitertanol + fuberidazole	$y = -110.4 + 15.14x_i + 7.67x_{ii}$	0.882	0.059
4	carboxin + thiram	$y = -34.2 + 12.98x_i + 3.29x_{ii}$	0.749	0.126
4	fluquinconazole + prochloraz	$y = -108.3 + 21.84x_i + 6.27x_{ii}$	0.950	0.025
6	untreated	$y = -99.6 + 18.66x_i + 4.18x_{ii}$	0.940	0.030
6	bitertanol + fuberidazole	$y = 2.8 + 21.31x_i + 5.31x_{ii}$	0.989	0.005
6	carboxin + thiram	$y = -25.0 + 12.98x_i + 8.58x_{ii}$	0.982	0.005
6	fluquinconazole + prochloraz	$y = 109.1 + 16.34x_i + 10.49x_{ii}$	0.864	0.068

x_i – average soil temperature; x_{ii} – average soil water content.
Seedlot **4** – 8 % infection; seedlot **6** – 88 % infection (Table 2; page 33).

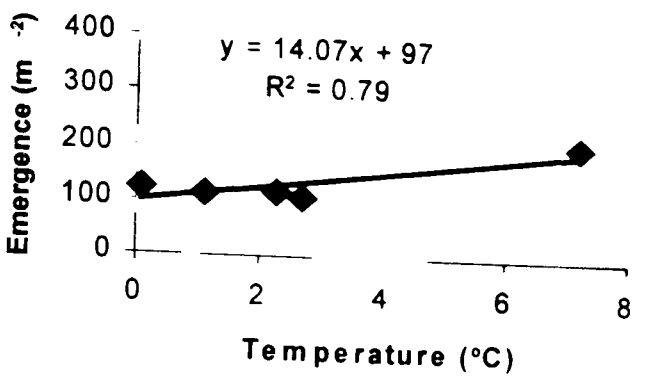
seeds (415 m^{-2}) and fluquinconazole + prochloraz treated seeds (388 m^{-2}). Plants from untreated seeds (335 m^{-2}) had significantly less shoots than plants from treated seeds ($P < 0.05$). The seedlot x seed treatment interaction was significant ($P < 0.001$). Plants from carboxin + thiram (416 m^{-2}), bitertanol + fuberidazole (372 m^{-2}) and fluquinconazole + prochloraz (348 m^{-2}) treated seeds of seedlot 4 produced significantly less ($P < 0.05$) shoots than seedlot 6 (carboxin + thiram treated seeds 457 m^{-2}; bitertanol + fuberidazole treated seeds, 458 m^{-2}; fluquinconazole + prochloraz 429 m^{-2}). There were no significant differences ($P = 0.207$) between seed treatments across all trials (Table 41).

Plants from trial 1 produced the most ears (336 m^{-2}), plants from trial 2 (259 m^{-2}) and trial 3 (258 m^{-2}) produced significantly less ($P < 0.05$) ears than trial 4 (284 m^{-2}) and trial 5 (293 m^{-2}). Plants from seedlot 6 produced significantly ($P < 0.05$) more ears (295 m^{-2}) than seedlot 4 (278 m^{-2}). Treatment of seed significantly increased ($P < 0.05$) ears m^{-2} above untreated seed. The seedlot x seed treatment interaction was significant ($P < 0.001$). Plants from carboxin + thiram (299 m^{-2}), bitertanol + fuberidazole (276 m^{-2}) and

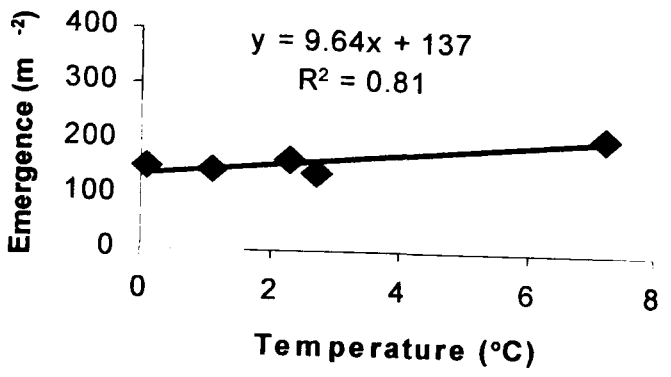
untreated seeds (a)



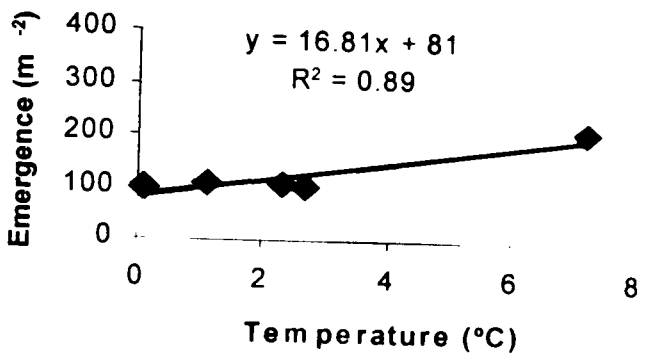
bitertanol + fuberidazole treated seeds (a)



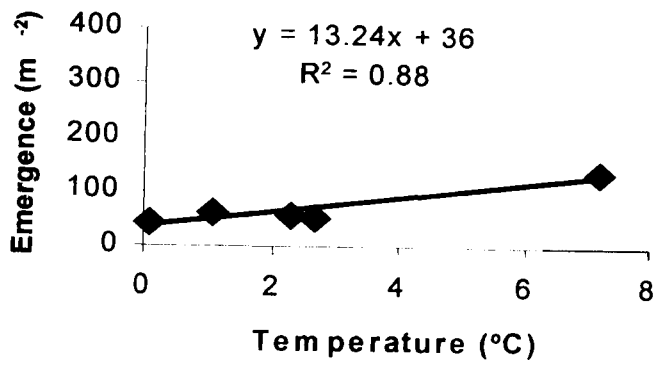
carboxin + thiram treated seeds (a)



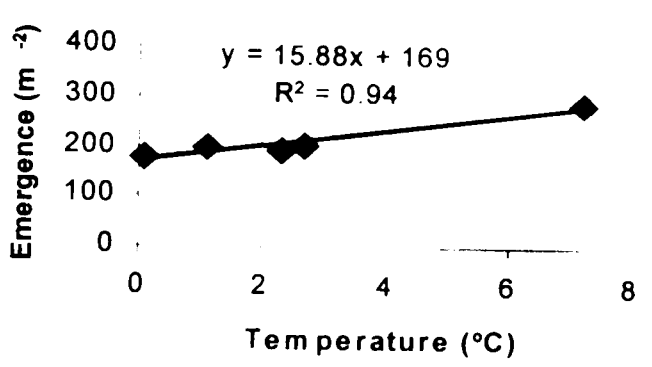
fluquinconazole + prochloraz treated seeds (a)



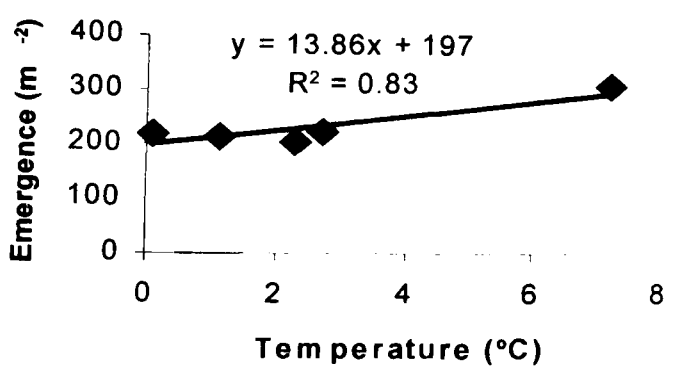
untreated seeds (b)



bitertanol + fuberidazole treated seeds (b)



carboxin + thiram treated seeds (b)



fluquinconazole + prochloraz treated seeds (b)

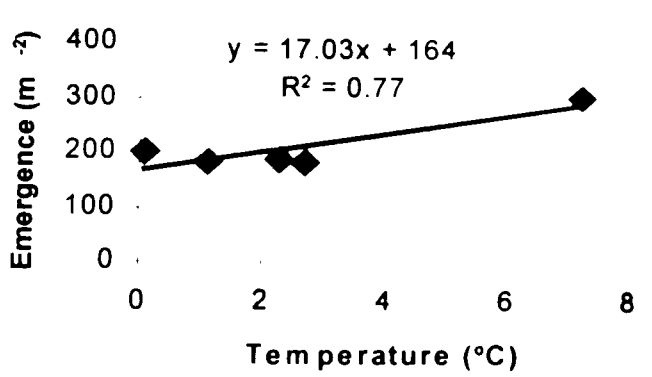


Figure 12. Effect of median temperature on days four and five after drilling on final emergence from two winter wheat seedlots (cv. Equinox) infected with *Microdochium nivale* with and without fungicide seed treatments in field trials at Harper Adams University College in 2000-2001.

a – seedlot **4** (8 % infection), **b** – seedlot **6** (88 % infection) (Table 2; page 32).

Table 41. Effect of seed treatments and drilling date on shoots m^{-2} at GS 39 and ears m^{-2} at GS 75 of plants from two winter wheat seedlots (cv. Equinox) infected with *Microdochium nivale* in field trials at Harper Adams University College in 2000-2001.

	tillers (m^{-2}) trial*						ears (m^{-2}) trial*					
treatment	**1**	**2**	**3**	**4**	**5**	**COMB**	**1**	**2**	**3**	**4**	**5**	**COMB**
8 % *M. nivale* infection (seedlot 4)												
untreated	428	322	291	355	344	348	302	248	235	270	268	265
bitertanol + fuberidazole	496	353	300	355	358	372	343	262	236	264	276	276
carboxin + thiram	493	370	355	418	444	416	341	274	262	295	321	299
fluquinconazole + prochloraz	458	286	304	359	330	348	332	238	244	281	258	271
88 % *M. nivale* infection (seedlot 6)												
untreated	439	307	258	298	305	321	308	226	195	223	248	240
bitertanol + fuberidazole	541	373	414	467	494	458	359	259	303	317	337	315
carboxin + thiram	497	412	427	469	482	457	344	288	295	313	326	313
fluquinconazole + prochloraz	487	379	409	427	442	429	360	278	296	308	315	311
LSD ($P < 0.05$)	72.1	58.2	54.2	45.9	46.8	54.3	42.3	36.2	40.8	33.6	38.4	37.0
SED	35.2	28.4	26.5	22.4	22.8	27.5	20.6	17.7	19.9	16.4	18.7	18.7
DF	28	28	28	28	28	140	28	28	28	28	28	140
%cv	11.6	12.8	12.1	9.0	9.0	11.0	9.7	10.8	12.2	9.1	10.1	10.4

* trial **1** - 10/2000, trial **2** - 10 01/2001, trial **3** - 25 01 2001, trial **4** - 09 02 2001, trial **5** - 21/02 2001.
Seedlot 4 - 8 % infection; seedlot 6 - 88 % infection (Table 2; page 33).

fluquinconazole + prochloraz (271 m^{-2}) treated seeds of seedlot 4 produced significantly less ($P < 0.05$) ears than seedlot 6 (carboxin + thiram treated seeds 313 m^{-2}; bitertanol – fuberidazole treated seeds, 315 m^{-2}; fluquinconazole + prochloraz 311 m^{-2}). The opposite trend occurred for untreated seeds (seedlot 4, 265 m^{-2}; seedlot 6, 240 m^{-2}). No significant differences ($P = 0.430$) in ears m^{-2} occurred between seed treatments across all trials (Table 41).

When data was pooled for all trials, shoots m^{-2} were significantly correlated to establishment ($y = 1.34x + 2.175$; $R^2 = 0.572$; $P < 0.001$). Ears m^{-2} were significantly correlated to shoots m^{-2} ($y = 1.55x - 49.7$; $R^2 = 0.850$; $P < 0.001$) and establishment ($R^2 = 0.663$; $P < 0.001$).

Treatment of seed significantly increased yield above untreated seeds ($P < 0.05$). There were no consistent differences between seed treatments across all trials (Table 42). Trial 1 (6.65 tonne ha^{-1}) gave the greatest yield and trials 2 (4.36 tonne ha^{-1}) and 3 (4.33 tonne ha^{-1}) the lowest yield ($P < 0.05$). The seed treatment x seedlot interaction was significant ($P < 0.001$). Seedlot 6 gave increased yields above seedlot 4 for all treated seeds, however, untreated seeds of seedlot 4 gave significantly increased ($P < 0.05$) yield above untreated seeds of seedlot 6. When data was pooled for all trials, yield was significantly correlated to ears m^{-2} ($R^2 = 0.702$; $P < 0.001$), shoots m^{-2} ($R^2 = 0.669$; $P < 0.001$) and establishment ($R^2 = 0.671$; $P < 0.001$) for each trial. Yield was also correlated to foot rot disease incidence at GS 39 ($R^2 = 0.609$; $P < 0.001$) and GS 75 ($R^2 = 0.597$; $P < 0.001$) for each trial. Seedlot 6 (47.9 g) produced a significantly ($P < 0.05$) heavier TGW than seedlot 4 (46.4 g). Trials 4 and 5 gave rise to significantly increased TGW ($P < 0.05$) above trials 1, 2 and 3 (Table 42). Carboxin + thiram seed treatment (46.6 g) significantly reduced ($P < 0.05$) TGW below fluquinconazole + prochloraz treated seeds (47.3 g) and untreated seeds (47.6 g).

Table 42. Effect of seed treatments and drilling date on yield (tonne ha^{-1}) and TGW (g) of plants from two winter wheat seedlots (cv. Equinox) with *Microdochium nivale* infection in field trials at Harper Adams University College in 2000-2001.

	yield (tonne ha^{-1}) trial*						TGW (g) trial*					
treatment	**1**	**2**	**3**	**4**	**5**	**COMB**	**1**	**2**	**3**	**4**	**5**	**COMB**
						8 % *M. nivale* infection (seedlot 4)						
untreated	6.58	4.44	4.16	5.06	5.44	5.14	45.7	44.7	45.8	48.3	48.5	46.6
bitertanol + fuberidazole	6.80	4.54	4.22	5.12	5.80	5.30	45.7	45.2	44.9	48.0	49.3	46.6
carboxin + thiram	6.34	4.64	4.68	5.44	5.42	5.30	45.5	43.8	44.9	47.1	48.0	45.8
fluquinconazole + prochloraz	6.84	4.62	4.32	5.42	5.60	5.36	46.8	44.9	45.7	47.9	47.4	46.5
						88 % *M. nivale* infection (seedlot 6)						
untreated	6.28	2.84	3.24	4.30	4.04	4.14	48.4	46.0	48.0	49.7	50.8	48.6
bitertanol + fuberidazole	6.70	4.56	4.74	5.36	6.00	5.47	45.9	45.3	46.4	48.5	51.0	47.4
carboxin + thiram	6.62	4.58	4.38	5.64	6.34	5.51	48.3	45.4	45.8	48.2	49.5	47.4
fluquinconazole + prochloraz	7.06	4.66	4.90	5.80	5.84	5.65	48.8	46.8	47.2	47.4	50.4	48.1
LSD ($P = 0.05$)	0.395	0.431	0.572	0.729	0.629	0.546	2.23	1.83	2.21	1.77	2.35	2.09
SED	0.193	0.210	0.280	0.356	0.307	0.276	1.09	0.89	1.08	0.86	1.15	1.06
DF	28	28	28	28	28	140	28	28	28	28	28	140
%cv	4.6	7.6	10.2	10.7	8.7	8.3	3.7	3.1	3.7	2.8	3.7	3.5

* **trial 1** drilled 25/10/2000, **trial 2** drilled 10/01/2001, **trial 3** drilled 25/01/2001, **trial 4** drilled 09/02/2001, **trial 5** drilled 21/02/2001.
Seedlot **4** = 8 % infection; seedlot **6** = 88 % infection (Table 2; page 33).

Seedling blight severity and foot rot disease incidence

Seedling blight data could not be statistically analysed due to a lack of normality. However, it is clear from Table 43 that seedling blight severity on seedlings from seedlot 4 was less than from seedlot 6 at GS 10-12 and GS 15-25. Seed treatments reduced disease severity compared to untreated seeds in all trials at GS 10-12 and GS 15-25. Seedling blight severity typically declined from GS 10-12 to GS 15-25.

Averaged across all trials, fluquinconazole + prochloraz significantly reduced foot rot disease incidence compared to all other seed treatments and untreated seeds of seedlot 4 at GS 39 ($P < 0.05$). Plants from untreated seeds had the highest incidence of foot rot at GS 39 (Table 44). Plants from seedlot 6 (88 % infection) had significantly ($P < 0.05$) higher incidences of foot rot disease (26.1 %) than plants from seedlot 4 (8 % infection; 14.1 %). Plants from trial 5 (13.0 %) had significantly reduced incidence of foot rot ($P < 0.05$). The seed treatment x seedlot x drilling date interaction was significant ($P = 0.006$). Seed treatments typically reduced foot rot disease incidence below untreated seeds across all drilling dates for seedlot 4 but not always for seedlot 6.

Averaged across all trials, plants from seedlot 6 (27.4 %) had significantly increased ($P < 0.05$) incidence of foot rot above plants from seedlot 4 (19.3 %) at GS 75 (Table 44). Plants from trial 2 (25.7 %), trial 4 (25.2 %) and trial 5 (26.8 %) had significantly ($P < 0.05$) increased foot rot incidence above plants from trial 1 (17.8 %). Plants from fluquinconazole + prochloraz treated seeds had significantly reduced foot rot disease incidences ($P < 0.05$). There was no correlation between foot rot incidence at GS 39 and GS 75.

Table 43. Effect of seed treatments and drilling date on seedling blight severity at GS 10-12 and GS 15-25 on seedlings from two winter wheat seedlots (cv. Equinox) infected with *Microdochium nivale* in field trials at Harper Adams University College in 2000-2001.

treatment	GS 10-12 trial*							GS 15-25 trial*						
	1	2	3	4	5	COMB		1	2	3	4	5	COMB	
8 % *M. nivale* infection (seedlot 4)														
untreated	8.8	10.0	4.8	3.7	5.6	6.6	(1.2)	8.6	2.0	1.0	1.8	0.4	2.8	(0.9)
bitertanol + fuberidazole	3.2	1.0	0.2	0.0	1.4	1.2	(0.3)	7.0	1.4	0.6	0.0	0.6	1.9	(0.9)
carboxin + thiram	3.2	1.2	0.4	0.8	1.8	1.5	(0.4)	10.6	3.0	0.0	0.2	0.0	2.8	(1.1)
fluquinconazole + prochloraz	4.2	0.0	0.0	0.0	1.8	1.2	(0.7)	1.0	0.0	0.6	0.0	0.4	0.4	(0.2)
88 % *M. nivale* infection (seedlot 6)														
untreated	46.0	50.4	22.2	31.9	36.1	37.3	(2.8)	23.9	5.7	0.3	0.7	0.7	6.2	(2.1)
bitertanol + fuberidazole	6.6	8.2	1.0	0.0	5.4	4.2	(0.9)	7.2	1.6	1.6	0.6	0.4	2.3	(0.7)
carboxin + thiram	21.6	8.8	7.2	13.4	3.6	10.9	(1.7)	8.2	1.4	0.8	0.4	0.2	2.2	(1.1)
fluquinconazole + prochloraz	2.2	1.2	0.0	0.0	0.0	0.7	(0.3)	0.2	0.0	0.0	0.0	1.0	0.2	(0.2)

* **trial 1** drilled 25/10/2000, **trial 2** drilled 10/01/2001, **trial 3** drilled 25/01/2001, **trial 4** drilled 09/02/2001, **trial 5** drilled 21/02/2001.
Numbers in parentheses represent SE.
Seedlot 4 8 % infection; seedlot 6 88 % infection (Table 2; page 33).

Table 44. Effect of seed treatments and drilling date on foot rot disease incidence (%) at GS 39 and GS 75 of plants from two winter wheat seedlots (cv. Equinox) infected with *Microdochium nivale* in field trials at Harper Adams University College in 2000-2001.

	GS 39 trial*							GS 75 trial*						
treatment	**1**	**2**	**3**	**4**	**5**	**COMB**		**1**	**2**	**3**	**4**	**5**	**COMB**	
						8 % *M. nivale* infection (seedlot 4)								
untreated	27.2	14.4	26.4	22.1	10.9	20.2	24.3[1]	18.6	26.7	17.3	32.0	20.0	22.9	26.9[1]
bitertanol + fuberidazole	14.4	12.8	20.6	15.2	7.5	14.1	20.1	10.7	20.0	9.3	13.4	18.7	14.4	20.1
carboxin + thiram	14.4	9.6	13.6	18.4	8.0	12.8	20.0	17.3	21.3	17.3	25.3	16.0	19.5	24.9
fluquinconazole + prochloraz	8.8	7.2	10.4	14.4	6.1	9.4	16.1	24.0	16.0	22.7	12.0	26.7	20.3	25.6
						88 % *M. nivale* infection (seedlot 6)								
untreated	8.8	40.3	46.9	30.7	27.2	30.8	31.8	24.0	37.3	26.7	33.5	48.0	33.9	35.2
bitertanol + fuberidazole	32.8	23.2	21.6	40.5	12.0	26.0	29.7	17.3	38.7	21.3	37.3	33.3	29.6	31.8
carboxin + thiram	20.8	25.6	39.5	43.7	20.8	30.1	32.3	21.3	28.0	34.6	29.3	33.3	29.3	31.5
fluquinconazole + prochloraz	27.2	10.4	20.0	19.2	11.2	17.6	23.9	9.3	17.3	20.0	18.7	18.7	16.8	22.3
	[2] LSD = 11.23, SED = 5.68, DF = 140, %cv = 36.2.							[3] LSD = 11.60, SED = 5.86, DF = 140, %cv = 34.0.						

* **trial 1** drilled 25/10/2000, **trial 2** drilled 10/01/2001, **trial 3** drilled 25/01/2001, **trial 4** drilled 09/02/2001, **trial 5** drilled 21/02/2001.

1 data angular transformed.

2 combined data analysis for GS 39.

3 combined data analysis for GS 75.

Seedlot **4** = 8 % infection; seedlot 6 = 88 % infection (Table 2; page 33).

Fungicide seed treatment efficacy and winter wheat performance in field trials during 2001-2002

Seedling emergence

Rate of emergence was significantly faster ($P < 0.05$) from the pathogen-free seedlot (seedlot 1; cv. Cadenza) when averaged across all drilling dates. Rate of emergence from carboxin + thiram treated seeds (0.0571 day^{-1}) was significantly faster ($P < 0.05$) than emergence from untreated (0.0549 day^{-1}) and fluquinconazole + prochloraz (0.0523 day^{-1}) treated seeds across all drilling dates. Fluquinconazole + prochloraz significantly slowed ($P < 0.05$) seedling emergence from both seedlots below all other seed treatments and untreated seed. Rate of emergence was significantly fastest ($P < 0.05$) from trial 1 (0.0947 day^{-1}) and slowest from trial 4 (0.0239 day^{-1}). The seedlot x seed treatment x drilling date interaction significantly affected rate of emergence ($P = 0.004$). All seed treatments and untreated seeds had similar rates of emergence from both seedlots across all four drilling dates. Significant differences ($P < 0.05$) only occurred in the first drilling (Table 45).

Final emergence from seedlot 1 (0 % infection; 310 plants m^{-2}) was significantly above ($P < 0.05$) seedlot 2 (29 % infection; 203 plants m^{-2}) averaged across all drilling dates (Table 46). Final emergence from untreated seeds was significantly lower ($P < 0.05$) than from treated seeds for both seedlots. Carboxin + thiram treated seeds gave rise to highest final emergence across all drilling dates. Final emergence was significantly reduced ($P < 0.05$) from trial 4. There was no significant correlation between rate of emergence and final emergence.

Establishment from seedlot 1 (257 plant m^{-2}) was significantly above ($P < 0.05$) seedlot 2 (179 plants m^{-2}). Averaged across all drilling dates, carboxin + thiram treated seeds (250 plants m^{-2}) gave significantly increased establishment above treated and untreated seeds across all drilling dates ($P < 0.05$). Bitertanol + fuberidazole (227 plants m^{-2}) and

Table 45. Effect of seed treatments and drilling date on rate of emergence (day^{-1}) from winter wheat seedlots (cv. Cadenza) with 0 % or 29 % *Microdochium nivale* infection in field trials at Harper Adams University College in 2001-2002.

	rate of emergence (day^{-1})				
	trial*				
treatment	**1**	**2**	**3**	**4**	**COMB**
	0 % *M. nivale* infection (seedlot 1)				
untreated	0.0974	0.0502	0.0538	0.0236	0.0563
bitertanol + fuberidazole	0.1000	0.0514	0.0559	0.0242	0.0579
carboxin + thiram	0.0991	0.0512	0.0553	0.0247	0.0576
fluquinconazole + prochloraz	0.0883	0.0481	0.0506	0.0233	0.0526
	29 % *M. nivale* infection (seedlot 2)				
untreated	0.0904	0.0479	0.0516	0.0240	0.0535
bitertanol + fuberidazole	0.0966	0.0487	0.0541	0.0234	0.0557
carboxin + thiram	0.0972	0.0507	0.0540	0.0245	0.0566
fluquinconazole + prochloraz	0.0879	0.0470	0.0504	0.0230	0.0521
LSD ($P < 0.05$)	0.00229	0.00137	0.00178	0.00068	0.00159
SED	0.00112	0.00067	0.00086	0.00033	0.00080
DF	28	28	28	28	140
%cv	1.9	2.1	2.6	2.2	2.3

*** trial 1** drilled 13/10/01, **trial 2** drilled 03/11/01, **trial 3** drilled 09/11/01, **trial 4** drilled 13/12/01.

For further information on the seedlots, see Table 2; page 33.

fluquinconazole + prochloraz treatments (232 plants m^{-2}) significantly increased ($P < 0.05$) establishment above untreated seeds (163 plants m^{-2}) for all drilling dates. Establishment was significantly reduced ($P < 0.05$) from trial 4 (Table 46). Establishment was significantly correlated to final emergence ($y = 18.67 + 0.777x$; $R^2 = 0.888$).

For seedlot 1, final emergence from untreated seeds was most closely correlated with median temperatures on day three after drilling ($y = 53.8 + 26.82x$; $R^2 = 0.923$; $P = 0.026$). For bitertanol + fuberidazole treated seeds of seedlot 1, median temperatures after drilling did not significantly correlate with final emergence. For carboxin + thiram treated seeds of seedlot 1, median temperature on day three ($y = 276.8 + 6.87x$; $R^2 = 0.869$; $P = 0.045$) and days three and four ($y = 283.1 + 6.39x$; $R^2 = 0.928$; $P = 0.035$) after drilling correlated best with final emergence. For fluquinconazole + prochloraz treated seeds of seedlot 1, median

Table 46. Effect of seed treatments and drilling date on final emergence (m^{-2}) at GS 10-12 and establishment (m^{-2}) at GS 15-25 from winter wheat seedlots (cv. Cadenza) with 0 % or 29 % *Microdochium nivale* infection in field trials at Harper Adams University College in 2001-2002.

	final emergence (m^{-2}) trial*					establishment (m^{-2}) trial*				
treatment	**1**	**2**	**3**	**4**	**COMB**	**1**	**2**	**3**	**4**	**COMB**
					0 % *M. nivale* infection (seedlot 1)					
untreated	330	334	270	99	258	302	264	199	77	210
bitertanol + fuberidazole	333	388	342	271	333	317	295	264	230	277
carboxin + thiram	349	351	328	289	329	328	286	256	248	280
fluquinconazole + prochloraz	327	371	347	235	320	289	290	272	200	263
					29 % *M. nivale* infection (seedlot 2)					
untreated	211	144	127	26	127	207	122	106	24	115
bitertanol + fuberidazole	229	216	224	141	203	225	182	180	124	178
carboxin + thiram	238	274	270	222	251	246	227	216	193	220
fluquinconazole + prochloraz	229	279	244	173	231	229	225	191	158	201
LSD ($P < 0.05$)	42.8	41.2	59.7	45.8	46.4	35.8	35.8	37.9	40.4	36.3
SED	20.9	20.1	29.2	22.4	23.4	17.5	17.5	18.5	19.7	18.3
DF	28	28	28	28	140	28	28	28	28	140
%cv	11.8	10.8	17.1	19.4	14.4	10.3	11.7	13.9	19.9	13.3

* trial 1 drilled 13/10/01, trial 2 drilled 03/11/01, trial 3 drilled 09/11/01, trial 4 drilled - 13/12/01.
For further information on the seedlots, see Table 2; page 33.

temperature on day 3 ($y = 221.1 + 12.97x$: $R^2 = 0.642$; $P = 0.128$) after drilling was most closely correlated with final emergence. Using multiple regression, median temperatures and soil water contents had good correlations with final emergence for both seedlots (Table 47).

Final emergence from untreated seeds of seedlot 2 correlated best with median temperature on days two and three ($y = 7.6 + 16.34x$; $R^2 = 0.981$; $P = 0.006$) and days three and four ($y = 0.9 + 17.48x$; $R^2 = 0.977$; $P = 0.008$) after drilling. Final emergence from bitertanol + fuberidazole treated seeds of seedlot 2 correlated best with median temperatures on day three ($y = 125.0 + 10.18x$; $R^2 = 0.952$; $P = 0.016$) after drilling. Final emergence from carboxin + thiram treated and fluquinconazole + prochloraz treated seeds of seedlot 2, did not correlate with median daily temperatures after drilling.

Subsequent plant productivity

Plants from seedlot 1 (437 m^{-2}) produced significantly ($P < 0.05$) more shoots m^{-2} than plants from seedlot 2 (401 m^{-2}). Plants from the third drilling date (467 m^{-2}) produced significantly ($P < 0.05$) more shoots m^{-2} than plants from trials 1 (399 m^{-2}), 2 (406 m^{-2}) and 4 (405 m^{-2}). Seed treatments had an inconsistent effect on shoots m^{-2} but significant increases above untreated seeds were seen in drilling dates 3 and 4 for both seedlots (Table 48).

Plants from seedlot 1 (425 m^{-2}) produced significantly ($P < 0.05$) more ears m^{-2} than plants from seedlot 2 (390 m^{-2}). Plants from the third drilling date (455 m^{-2}) produced significantly more ($P < 0.05$) ears m^{-2} than plants from trials 1 (365 m^{-2}), 2 (400 m^{-2}) and 4 (411 m^{-2}). No seed treatment significantly increased ears m^{-2} above untreated seeds consistently for either seedlot (Table 48). Ears m^{-2} was correlated to shoots m^{-2} ($y = 75.2 + 0.793x$; $R^2 = 0.613$; $P < 0.001$) using drilling date as a grouping.

Table 47. Multiple regression analysis for the effect of temperature and soil water content on final emergence from winter wheat seedlots (cv. Cadenza) with 0 % or 29 % *Microdochium nivale* infection in field trials at Harper Adams University College in 2001-2002.

seedlot	seed treatment	days after drilling	equation	R^2	*P*
1	untreated	15-20	$y = 614.0 - 11.02x_i - 18.45x_{ii}$	0.988	0.063
1	bitertanol + fuberidazole	0-10	$y = 738.6 + 11.67x_i - 23.57x_{ii}$	0.947	0.133
1	carboxin + thiram	0-10	$y = 460.5 + 7.88x_i - 8.97x_{ii}$	0.993	0.053
		15-20	$y = 437.1 - 2.30x_i - 5.33x_{ii}$	0.997	0.033
1	fluquinconazole + prochloraz	10-20	$y = 769.8 + 5.70x_i - 22.36x_{ii}$	1.000	0.012
2	untreated	0-10	$y = 168.0 + 19.06x_i - 8.19x_{ii}$	0.956	0.124
		10-20	$y = 55.6 + 20.16x_i - 2.22x_{ii}$	0.981	0.080
2	bitertanol + fuberidazole	10-20	$y = 328.1 + 8.25x_i - 8.06x$	0.906	0.176
2	carboxin + thiram	10-20	$y = 517.9 - 0.63x_i - 12.17x_{ii}$	0.914	0.168
2	fluquinconazole + prochloraz	0-10	$y = 601.9 + 10.56x_i - 21.52x_{ii}$	0.877	0.200
		10-20	$y = 614.8 + 2.51x_i - 18.42x_{ii}$	0.949	0.132

x_i – average soil temperature; x_{ii} – average soil water content.
Seedlot **1** (cv. Cadenza; 0 % infection), seedlot **2** (cv. Cadenza; 29 % infection) see Table 2; page 33 for more details.

Plants from seedlot 1 (8.60 tonne ha^{-1}) produced significantly ($P < 0.05$) higher yields than plants from seedlot 2 (8.17 tonne ha^{-1}). Plants from the third trial (9.58 tonne ha^{-1}) produced significantly higher ($P < 0.05$) yield above trials 1 (8.43 tonne ha^{-1}) and 2 (8.18 tonne ha^{-1}). Trial 4 produced significantly the lowest yield ($P < 0.05$). Plants from untreated seeds produced the lowest yields ($P < 0.05$). Effects of seed treatments on yield were especially apparent in later drilled trials. Untreated seeds of both seedlots produced lowest yields in trial 4 (Table 49). No consistent trends occurred between seedlots across all drilling dates. Yield was correlated to establishment ($R^2 = 0.528$; $P < 0.001$) within individual trials. Drilling date, seed treatment, seedlot and their interactions had no significant effects on TGW (Table 49).

Table 48. Effect of seed treatments and drilling date on shoots (m^{-2}) at GS 39 and ears (m^{-2}) at GS 75 for plants from winter wheat seedlots (cv. Cadenza) with 0 % or 29 % *Microdochium nivale* infection in field trials at Harper Adams University College in 2001-2002.

	tillers (m^{-2}) trial*					ears (m^{-2}) trial*				
treatment	**1**	**2**	**3**	**4**	**COMB**	**1**	**2**	**3**	**4**	**COMB**
					0 % *M. nivale* infection (seedlot 1)					
untreated	417	459	449	404	432	382	453	472	425	433
bitertanol + fuberidazole	418	433	489	415	439	393	429	470	429	430
carboxin + thiram	449	421	503	425	449	396	411	472	408	422
fluquinconazole + prochloraz	398	401	488	421	427	369	389	474	428	415
					29 % *M. nivale* infection (seedlot 2)					
untreated	381	378	402	374	384	340	380	473	401	399
bitertanol + fuberidazole	375	398	477	395	411	325	391	429	391	384
carboxin + thiram	381	374	491	409	414	353	364	441	405	391
fluquinconazole + prochloraz	372	382	435	398	397	361	380	409	399	387
LSD ($P = 0.05$)	47.5	47.9	93.7	NS	NS	44.2	55.8	NS	NS	NS
SED	23.2	23.3	45.7	35.2	33.3	21.6	27.2	43.4	42.6	35.1
DF	28	28	28	28	140	28	28	28	28	140
$\%_{cv}$	9.2	9.1	15.5	13.8	12.6	9.4	10.8	15.1	16.4	13.6

* **trial 1** drilled 13/10/01, **trial 2** drilled 03/11/01, **trial 3** drilled 09/11/01, **trial 4** drilled - 13/12/01.
For further information on the seedlots, see Table 2; page 33

Seedling blight severity and foot rot disease incidence

Seedling blight data could not be statistically analysed due to a lack of normality. Seed treatments reduced seedling blight severity at GS 10-12 and GS 15-25 below untreated seeds (Table 50). Fluquinconazole + prochloraz treatment most effectively reduced seedling blight at GS 10-12 over all drilling dates. At GS 10-12, seedlings from seedlot 2 (11.59 %) were more diseased than seedlings from seedlot 1 (2.94 %). At GS 15-25, seedlings from seedlot 2 (5.36 %) were more diseased than seedlings from seedlot 1 (2.93 %). Disease severity on untreated seeds was much reduced at GS 15-25 compared to GS 10-12 for seedlot 2.

Foot rot disease incidence was significantly lower ($P < 0.05$) on plants from seedlot 1 (53 %) than seedlot 2 (67 %) at GS 39 (Table 51). Highest foot rot disease incidence occurred in trial 3 ($P < 0.05$). Averaged across all drilling dates and seedlots, fluquinconazole + prochloraz treatment significantly reduced ($P < 0.05$) foot rot disease incidence at GS 39. Foot rot disease incidence in trials generally increased between GS 39 and GS 75.

Foot rot disease incidence was significantly lower ($P < 0.05$) on plants from seedlot 1 (68 %) than seedlot 2 (82 %) at GS 75 (Table 51). Foot rot disease incidence at GS 75 was significantly ($P < 0.05$) lower on trial 1 (64 %) than trials 2 (83 %), 3 (77 %) and 4 (75 %). Fluquinconazole + prochloraz treatment significantly reduced ($P < 0.05$) foot rot disease incidence at GS 75. Plants from untreated seeds had significantly higher ($P < 0.05$) foot rot incidence than plants from treated seeds. No correlation occurred between foot rot disease incidence at GS 39 and GS 75.

Table 49. Effect of seed treatments and drilling date on yield (tonne ha^{-1}) and TGW (g) for plants from winter wheat seedlots (cv. Cadenza) with 0 % or 29 % *Microdochium nivale* infection in field trials at Harper Adams University College in 2001-2002.

	yield (tonne ha^{-1})					TGW (g)					
	trial*					trial*					
treatment	**1**	**2**	**3**	**4**	**COMB**	**1**	**2**	**3**	**4**	**COMB**	
					0 % *M. nivale* infection (seedlot 1)						
untreated	8.72	8.16	8.92	5.26	7.77	54.2	50.5	50.3	47.7	50.7	7.11[1]
bitertanol + fuberidazole	8.76	8.00	10.10	8.35	8.80	54.5	50.2	49.2	49.3	50.8	7.12
carboxin + thiram	8.85	8.42	9.71	8.38	8.84	55.1	49.5	48.6	49.2	50.6	7.11
fluquinconazole + prochloraz	8.28	9.01	9.92	8.73	8.99	53.9	51.8	49.3	48.7	50.9	7.13
					29 % *M. nivale* infection (seedlot 2)						
untreated	8.03	7.36	9.03	3.84	7.07	55.6	52.2	49.7	47.3	51.2	7.15
bitertanol + fuberidazole	8.03	7.69	9.10	7.94	8.19	55.8	49.7	50.6	49.4	51.4	7.16
carboxin + thiram	8.52	8.10	9.81	8.15	8.64	54.2	48.5	48.3	48.8	49.9	7.06
fluquinconazole + prochloraz	8.28	8.73	10.05	8.12	8.80	53.3	51.2	50.5	49.7	51.2	7.15
LSD ($P < 0.05$)	0.769	1.090	NS	0.716	1.116	NS	NS	NS	NS	-	NS
SED	0.375	0.531	0.849	0.350	0.563	1.07	1.27	1.73	1.40	-	0.099
DF	28	28	28	28	140	28	28	28	28	-	140
%cv	7.0	10.3	14.0	7.5	10.6	3.1	4.0	5.5	4.5	-	2.2

trial 1 drilled 13/10/01, trial 2 drilled 03/11/01, trial 3 drilled 09/11/01, trial 4 drilled 13/12/01.
1 data square root transformed. For further information on the seedlots, see Table 2: page 33.

Table 50. Effect of seed treatments and drilling date on seedling blight disease severity at emergence (GS 10-12) and establishment (GS 15-25) on seedlings from winter wheat seedlots (cv. Cadenza) with 0 % or 29 % *Microdochium nivale* infection in field trials at Harper Adams University College in 2001-2002.

	GS 10-12 trial*						GS 15-25 trial*					
treatment	1	2	3	4	COMB		1	2	3	4	COMB	
0 % *M. nivale* infection (seedlot 1)												
untreated	3.4	2.8	4.2	7.8	3.8	(1.3)	13.2	1.4	1.6	5.1	5.4	(2.0)
bitertanol + fuberidazole	0.2	2.4	2.0	10.2	1.7	(1.1)	4.0	0.2	1.2	1.0	1.9	(0.6)
carboxin + thiram	0.4	2.2	1.0	3.4	1.4	(0.5)	1.6	0.8	1.6	1.4	1.4	(0.3)
fluquinconazole + prochloraz	0.8	0.6	1.6	4.0	1.2	(0.6)	11.8	0.4	1.0	0.6	4.4	(1.5)
29 % *M. nivale* infection (seedlot 2)												
untreated	26.2	32.2	28.4	35.2	28.1	(1.5)	1.2	10.4	12.4	17.2	7.9	(1.8)
bitertanol + fuberidazole	2.2	13.6	8.6	12.8	8.0	(1.4)	10.0	5.4	2.6	3.8	5.9	(1.6)
carboxin + thiram	1.8	4.8	4.0	7.4	3.6	(1.0)	7.4	2.4	5.2	4.6	5.0	(1.2)
fluquinconazole + prochloraz	0.6	1.5	0.6	5.6	1.0	(0.6)	1.2	0.0	0.0	2.0	0.5	(0.4)

* **trial 1** drilled 13/10/01, **trial 2** drilled 03/11/01, **trial 3** drilled 09/11/01, **trial 4** drilled 13/12/01.
Numbers in parentheses represent SE.
For further information on the seedlots, see Table 2; page 33.

Table 51. Effect of seed treatments and drilling date on foot rot disease incidence (%) at GS 39 and GS 75 from winter wheat (cv. Cadenza) seedlots with 0 % or 29 % *Microdochium nivale* infection in field trials at Harper Adams University College in 2001-2002.

	GS 39 trial*						GS 75 trial*					
treatment	**1**	**2**	**3**	**4**	**COMB**		**1**	**2**	**3**	**4**	**COMB**	
					0 % *M. nivale* infection (seedlot 1)							
untreated	44	41	76	48	52	46[1]	47	92	73	71	71	59[1]
bitertanol + fuberidazole	55	42	66	62	57	49	54	87	72	78	73	60
carboxin + thiram	59	33	75	58	57	49	58	71	70	58	65	54
fluquinconazole + prochloraz	49	26	61	59	49	44	60	66	63	60	62	52
					29 % *M. nivale* infection (seedlot 2)							
untreated	24	90	89	92	74	64	83	92	100	84	93	78
bitertanol + fuberidazole	21	86	85	83	69	59	77	90	84	87	84	69
carboxin + thiram	30	74	84	82	67	56	74	90	82	79	81	65
fluquinconazole + prochloraz	48	45	81	52	56	49	58	79	74	70	70	57

[2] LSD = 12.9, SED = 6.5, DF = 140, %cv = 19.8 [3] LSD = 10.6, SED = 5.4, DF = 140, %cv = 13.7

* **trial 1** drilled 13/10/01, **trial 2** drilled 03/11/01, **trial 3** drilled 09/11/01, **trial 4** drilled 13/12/01.

1 data angular transformed

2 combined data analysis for GS 39.

3 combined data analysis for GS 75.

For further information on the seedlots, see Table 2; page 33.

DISCUSSION

Accurate recording of soil water content and soil temperature during this investigation have shown that seedbed conditions during seedling emergence are more important than drilling date *per se* for *M. nivale* seedling blight. However, drilling date did significantly affect final emergence and yield in both years of trials at Harper Adams University College. This is probably through repeated drillings between October and December-January providing a wide range of temperatures and soil water contents. Mesterhazy (1985) investigated the effect of location on *Fusarium* seedling blight in field trials between 1980-3. Significant cultivar x trial location interactions occurred in two years for *F. graminearum* seedling blight, in one year for *F. culmorum* seedling blight, and in one year for general *Fusarium* seedling blight from surface-inoculated seeds. An effect of sowing date on infection of sugar beet by *Polymyxa betae* has also been demonstrated (Blunt *et al.*, 1992).

There was no trial location x seed treatment interaction in this investigation. Only Gaudet *et al.* (1989) and Paveley & Davies (1994) have documented the effect of trial location on seed treatment performance. Gaudet *et al.* (1989) reported a significant location x seed treatment interaction for control of common bunt at four spring wheat sites over three years and two winter wheat sites over five years. Paveley & Davies (1994) highlighted variable final emergence from a winter barley seedlot treated with six fungicide seed treatments grown at six sites and a winter wheat seedlot treated with six fungicide seed treatments grown at nine sites in the UK. These findings could be related to soil temperature differences between sites but seedbed conditions were not recorded. In addition, the biology and control of *Fusarium* seedling blight and bunt are very different.

The significant linear relationship between final emergence and soil temperature after drilling in three years of field trials implies specific temperatures during emergence are very important determinants of final emergence. In two years of field trials at Harper Adams University College there was no such relationship for soil water contents and final emergence. However, multiple linear regression did show for the first time, that final emergence from seeds infected with *M. nivale* is influenced by specific periods of temperature and soil water contents. Khah *et al.* (1986) reported that final emergence from two spring wheat (cv. Timmo) seedlots was linearly related to mean soil temperature, between 7 and 11 °C and inversely related to soil moisture content between 12.1 and 15.5 % w/w. However, it is still unclear whether temperatures and soil water contents are affecting *M. nivale*, the seedling or the host-pathogen interaction.

Median temperature on day five after planting was significantly correlated with final emergence from treated and untreated seeds of seedlot 9 (cv. Riband; 56 % infection). Median temperature on days four and five after planting was significantly correlated with final emergence from treated and untreated seeds of seedlot 4 (cv. Equinox; 8 % infection) and seedlot 6 (cv. Equinox; 88 % infection). Final emergence from seedlot 1 (cv. Cadenza; 0 % infection) and seedlot 2 (cv. Cadenza; 29 % infection) was inconsistently related to temperature. However, when significant correlations did occur between final emergence and temperature for untreated seeds and treated seeds, it was for median temperature on days two to four after drilling. Reasons for this, are not readily apparent. It is also interesting to note that for seedlot 1 but not for seedlot 2, there was a relationship between temperature and final emergence for carboxin + thiram treated seeds and fluquinconazole + prochloraz, whilst for untreated seeds of seedlots 1 and 2 there was a consistent relationship. This may be attributed to differential seed treatment effects on the two seedlots or unquantified interactions between the soil, seed and seed treatment. Limited controlled environment investigations have been conducted into the effects of temperature

and soil water on seed treatment performance. Organomercury efficacy was not significantly different against seed surface-borne and seed-borne *M. nivale* between soil water contents of 8.9 and 23.9 % (Millar & Colhoun, 1969b) and soil-borne *F. culmorum* between soil water contents of 26 and 31 % (Bateman, 1977). In controlled environment studies, temperature has been shown to affect seed treatment performance measured by final emergence from *M. nivale* infected seedlots (Hare *et al.* 1995). Fluctuating temperatures and soil water content effects may explain reports of inconsistent seed treatment performance in field conditions (Morris *et al.*, 1994; Cox & Mussard, 1994).

Rate of emergence was correlated with final emergence in only one year. Hare *et al.* (1995) reported a good correlation between rate of emergence and final emergence from *M. nivale* infected seedlots in pot trials. The lack of such a correlation in these trials could be attributed to fluctuating temperatures and soil water contents, extremes of temperatures and soil water contents or the presence of soil-borne pathogens. Seed germination, in addition to the extent of *M. nivale* infection, affected emergence. In 2001-2, pathogen-free seeds of high germination emerged faster than diseased seeds. The opposite trend was seen in 2000-1. This highlights the importance of seed vigour in determining final field emergence. Nine seed vigour parameters of six barley cultivars were significantly correlated to rate of emergence in a field trial in 1972 (Ching *et al.*, 1977). Marshall & Naylor (1985) also recorded reduced final field emergence from low vigour seedlots of nine Italian ryegrass (cv. RvP) seedlots.

Seed vigour in addition to *M. nivale* infection affected plant productivity. Seedlot 6 (cv. Equinox; 88 % infection; 88 % germination) gave rise to more productive plants than seedlot 4 (cv. Equinox; 8 % infection; 78 % germination) in 2000-1. Good correlations occurred between establishment (m^{-2}) and shoots m^{-2}, ears m^{-2} and yield in both years for all seedlots used. This implies establishment is a critical determinant of yield. This is in

agreement with the findings of Spink *et al.* (2000) and Gooding *et al.* (2002) who used pathogen-free winter wheat seedlots.

Seed treatments significantly increased final emergence and establishment in all trials. Seed treatments also had beneficial effects on subsequent plant growth in both years of trials at Harper Adams University College. Seed treatments had more affect on shoots m^{-2} and ears m^{-2} for Equinox seedlots than Cadenza seedlots. This is probably due to a combination of low germination and increased *M. nivale* seed-borne infection for the Equinox seedlots in 2000-1. In 2001-2, seedlot affected crop productivity. This was probably due to the *M. nivale* infection of the respective seedlots. Caution must be applied to the results obtained in this trial because the extent of seed infection in seedlots 2 and 6 was far above the UK treatment threshold. However, *M. nivale* infection above 50 % can occur in certified seedlots in the UK (Cockerell & Rennie, 1995).

Seed treatments typically increased yields in both years of field trials at Harper Adams University College, especially for later drilled trials through increased establishment above untreated seeds. This is the first report of seed treatments increasing establishment and yield for pathogen-free seedlots, especially for later drillings (November to February). This contradicts the findings of Paveley & Davies (1994). It is likely the seed treatments increased emergence in later drilled trials by protecting the seeds from soil-borne seedling blight pathogens. Hare *et al.* (1995) demonstrated a relationship between rate of emergence and final emergence from naturally infected *M. nivale* seedlots in controlled environment trials. It is probable a similar relationship would occur for soil-borne inoculum. Therefore, the potential for seedling infection from soil-borne inoculum would be greater in later drilled trials due to severely reduced seedling growth. Seed treatments would protect emerging seedlings under these conditions increasing emergence above untreated seeds.

The effects of seed treatment on yield are related to the extent of seed-borne *M. nivale* infection, which affects final emergence. From fifteen field trials between 1967 and 1971 with oat seedlots infected with *M. nivale* and *P. avenae*, good correlations occurred between establishment, ear populations and yield (Richardson, 1974). In field trials between 1979 and 1983 with 36 winter wheat seedlots, seed treatments only significantly increased yield for seedlots with seed-borne *M. nivale* infection above 10 %, implying plants cannot compensate for severe seedling losses (Richardson, 1986). Work by Noon & Jackson (1992) demonstrated increased establishment was responsible for yield increases from treated seeds for a seedlot with over 70 % *M. nivale* infection. Yield increases from treated seeds were greater over untreated seeds at the site with increased establishment over untreated seeds. Hare (1997) reported a similar occurrence in a field trial (1993-4) at two UK sites. Establishment from untreated seeds was much reduced (15.1 to 2.5 plants m^{-1}) at the site, which gave significant yield differences between treated and untreated seeds. It is also possible that surviving seedlings from untreated seedlots will be unable to compensate fully for killed seedlings.

This is the first demonstration of an interaction between drilling date and seed treatments affecting all aspects of winter wheat growth and yield. The close relationship between the extent of seed-borne *M. nivale* infection, establishment and yield was demonstrated in field trials for wheat (Humphreys *et al.*, 1995) and oats (Humphreys *et al.*, 1998). Timing of drilling has also been shown to affect wheat yields. Grain yield declined from 9.2 to 5.5 tonne ha^{-1} as plant populations declined from 336 to 13 m^{-2} in field trials between 1996-7, 1997-8 and 1998-9 with four pathogen-free winter wheat seedlots and three drilling dates from September to November (Spink *et al.*, 2000). Inadequate plant compensation may occur due to drilling into poor seedbeds or late drilling reducing the opportunity for compensation to occur.

Crop performance will also be influenced by environmental conditions determining disease pressure, infection and severity during the season. In both years of field trials at Harper Adams University College, the first drilled trials had the lowest incidence of foot rot. This may again indicate a link between seedling blight and foot rot. There was no correlation between foot rot disease incidence at GS 39 and GS 75 in this investigation. This may imply soil-borne inoculum was having an influence in both years because the severity of disease symptoms on seedlings from treated seeds was low. Seed treatments typically had little effect on foot rot incidence caused by *F. culmorum* and *M. nivale* at GS 30-31, GS 45 and GS 75 in 1993/4 at two sites in the UK (Hare, 1997). This is probably because seed treatment concentrations were much reduced at the time of disease challenge.

CHAPTER 9

General Discussion & Further Work

GENERAL DISCUSSION

Microdochium nivale seedling blight is an important disease of winter wheat in the UK. Large yield reductions can arise following severe pre-emergence seedling death. Seedling blight may also provide a source of inoculum for subsequent foot rot disease or ear blight epidemics. Seedling blight is usually controlled by fungicide seed treatments. However, current cost cutting measures in UK agriculture and environmental considerations mean approaches are required to target seed treatments to conditions under which severe seedling blight is most likely to occur. To meet this aim, greater knowledge of the epidemiology and control of *Fusarium* seedling blight is required.

Effect of seed-borne *Microdochium nivale* infection on seedling growth

Microdochium nivale often had detrimental effects on the initial phases of seedling growth (imbibition and germination) under a range of temperatures and soil water contents (Chapter 4). Seed-borne *M. nivale* tended to adversely affect imbibition and germination more at 5 °C and reduced soil water contents (< -1 MPa) than 15 °C and higher soil water contents (> -1 MPa). This confirms the findings of Cristani (1992) that *M. nivale* seedling blight is more severe under conditions in which seedling growth is slower. However, effects of *M. nivale* infection on seedling growth were not consistent across all seedlots, as shown by the interactions between water potential and temperature. This may be due to the extent of seed infection and cultivar differences in susceptibility to *Fusarium* seedling blight as highlighted by Arsenuik *et al.* (1993) and Pavlova & Srobarova (1997). It appeared that post-emergent disease severity was inversely related to the rate of seedling emergence. First leaf lengths were shorter from seedlings with severe disease symptoms than healthy seedlings or seedlings with less severe seedling blight. A slower rate of emergence may increase the opportunity for severe seedling blight to occur. Conversely, it could also be possible that severe seedling blight slows seedling germination and

emergence. This is an area that requires further investigation. A relationship between the amount of inoculum on individual seeds and subsequent seedling blight severity remains unproven. Millar & Colhoun (1969b) demonstrated that the inoculum load on surface-inoculated seeds can influence seedling blight severity although it is not apparent whether the same trend arises for naturally infected seeds.

Two groups of *M. nivale*; var. *majus* and var. *nivale* have been identified (Lees *et al.*, 1995). *Microdochium nivale* var. *majus* has been documented to be the more widely distributed in winter wheat crops in the UK (Parry *et al.*, 1995; Turner *et al.*, 1999). It has been speculated that this maybe due to increased *M. nivale* var. *majus* pathogenicity to winter wheat (Simpson *et al.*, 2000). In this investigation, the *in vitro* base temperatures for growth of var. *majus* isolates were lower than for var. *nivale* isolates. However, var. *majus* isolates had a faster growth rate than var. *nivale* isolates (Chapter 5). Therefore, it appears that the increased pathogenicity of var. *majus* to winter wheat seedlings reported by Hare (1997) cannot be explained by its increased growth at reduced temperatures giving it a competitive advantage over var. *nivale*.

Microdochium nivale biomass in seeds and seedlings was not substantially increased at GS 01 or GS 10 under low temperatures or reduced soil water contents measured by quantitative PCR (Chapter 5). However, *M. nivale* DNA did increase when seeds of two seedlots naturally infected with *M. nivale* were imbibed in a -4 MPa solution for up to 50 days. Analysing the rate of seedling imbibition and rate of emergence along with the growth of *M. nivale* both *in vitro* and *in planta* biomass ratios did not reveal any favourable ratios for *M. nivale* growth under conditions hindering seedling growth. This implies that observations made by Hare *et al.* (1995) that the rate of emergence is strongly related to final emergence of wheat plants growth from *M. nivale* infected seedlots, is

related to seedling growth and not *M. nivale* growth. At present, the process of infection of seedlings from seed-borne *M. nivale* infection is unknown.

The timing and severity of freezing significantly affected pre-emergent and post-emergent seedling blight severity from seed-borne *M. nivale* infection (Chapter 6). Pre-emergent exposure to -5 °C reduced final emergence most severely. This suggests that exposure to -5 °C slowed the rate of emergence more than exposure to 0 °C, increasing the window of opportunity for *M. nivale* infection. This confirms the observations made by other workers who noted that *M. nivale* causes more severe damage to cereal seedlings at freezing temperatures (Holmes & Channon, 1975; Perry, 1986). However, it is also possible that seed-borne *M. nivale* has more severe effects on seedling growth under freezing temperatures. Post-emergent freezing had a lesser effect on seedling blight severity. Further work is required to elucidate the mechanisms behind the seedling and *M. nivale* responses to freezing.

Effect of temperature and soil water content on *Microdochium nivale* seedling blight

Previous studies (Millar & Colhoun, 1969b; Hare *et al.*, 1995) have demonstrated that increased seedling blight severity occurs under conditions of low temperature and reduced soil water regimes. In this investigation, seedling growth was slower at 5 than 15 °C. However, the effect of seed-borne *M. nivale* infection and water potential was inconsistent, as were the interactions between temperature, water potential and *M. nivale* infection. It might be suggested that slower seedling growth increases the opportunity for *M. nivale* infection. It might also be expected that earlier infection by *M. nivale* will lead to more severe seedling blight but results from this investigation cannot confirm this hypothesis. Seed-borne *M. nivale* tended to more adversely affect the initial phases of seedling growth (imbibition and germination) at 5 °C and reduced soil water contents (< -1 MPa) (Chapter 4). However, subsequent investigations into *M. nivale* and seedling growth rate ratios

failed to explain why seedling blight severity was increased under these conditions (Chapter 5).

Field trials with multiple drilling dates in this investigation demonstrated that ranges of temperatures and soil water contents between drilling and 30 days post-drilling, have important effects on final emergence of winter wheat from seedlots heavily infected with *M. nivale* or with little or no *M. nivale* infection (Chapter 8). Specific effects appeared to depend on the temperature and soil water content range. Similar temperature and soil water content trends affecting final emergence were observed for untreated pathogen-free and *M. nivale* infected seedlots although these were not always significant and did not always occur for all fungicide seed treatments. Therefore, it is probable that the *M. nivale*-seedling interaction is the most important determinant of final emergence from infected seedlots. However, final emergence was very poor from a seedlot with low seed germination in conjunction with low seed-borne *M. nivale* infection (seedlot 4: cv. Equinox; 8 % infection; 78 % germination) in 2000-2001. This highlights the importance of seed vigour, in addition to the extent of seed-borne *M. nivale* infection on final emergence.

Effect of seed-borne *Microdochium nivale* on subsequent winter wheat growth and yield

Seed-borne *M. nivale* was shown to cause foot rot disease, even in the absence of seedling blight symptoms (Chapter 7). Severe seedling blight adversely affected subsequent plant growth and increased the extent of stem colonisation. Similar trends of reduced plant growth were observed in field trials (Chapter 8). This confirms similar observations on wheat (Humphreys *et al.*, 1995) and oat (Humphreys *et al.*, 1998) crops from seedlots infected with *M. nivale*. This investigation has further confirmed that *M. nivale* is a commercially important pathogen of winter wheat in the UK. However, it is likely that *M.*

nivale will have less severe effects in commercial seedlots which are usually less severely infected. This is an area for further research with the aim of determining thresholds of seed infection that are more closely linked to seed bed conditions.

Chemical control of *Microdochium nivale* seedling blight of winter wheat

In this study, fungicide seed treatments provided robust control of *M. nivale* seedling blight under a range of seedbed conditions (Chapter 8). Carboxin + thiram seed treatment provided the most robust control of seed-borne *M. nivale* over all drilling dates. Soil moisture and temperature at drilling and up to 30 days after drilling were important determinants of final emergence from treated seeds of *M. nivale* infected and pathogen-free seedlots. Establishment was the major determinant of yield in two years of field trials at Harper Adams confirming previous observations made by Richardson (1986) and Spink *et al.* (2000). The close relationship between establishment and yield highlights the importance of seed treatments for increasing establishment from seedlots with *M. nivale* infection above untreated seeds. However, yield from carboxin + thiram treated seeds was not always the highest despite the highest establishment, implying that other factors such as soil-borne inoculum, have important effects during the season. Seed germination, in addition to the extent of *M. nivale* infection had an important effect on establishment. Reduced final emergence under field conditions from low vigour seeds has been recorded for barley (Ching *et al.*, 1977) and ryegrass (Marshall & Naylor, 1985) in the absence of seedling pathogens.

Effect of seed treatments on subsequent winter wheat growth and yield

Carboxin + thiram seed treatment reduced foot rot disease incidence and stem colonisation from seed-borne *M. nivale* in pot trials (Chapter 7). This appeared to be due to reductions in seedling blight, possibly lowering inoculum amounts available for subsequent stem infections. Carboxin + thiram seed treatment also increased plant productivity, implying

M. nivale can adversely affect plant growth after GS 15-25. However, a similar trend for seed treatments consistently reducing foot rot incidence across all drilling dates was not evident in two years of field trials (Chapter 8). It is probable that this is due to the presence of soil-borne inoculum under field conditions but this has yet to be proven. Fluquinconazle + prochloraz appeared to be the most effective seed treatment in reducing foot rot incidence at GS 75. Foot rot incidence is likely to have important effects on yield, as shown in Chapter 7, however the correlation between establishment and yield was greater. In the field trials in this investigation, foot rot severity was not assessed. It is likely foot rot severity and incidence will affect yield and this requires further investigation.

Effects of seed treatments on seedling emergence, subsequent plant growth and yield from winter wheat seedlots with low or no *Microdochium nivale* infection

The seed treatments tested had beneficial effects at all stages of plant growth measured in 2001-2002 for a seedlot (cv. Cadenza) with no seed-borne infection in comparison to untreated seeds, especially in later drilled trials. In addition, beneficial effects of seed treatments were observed on a seedlot (cv. Equinox) with 8 % seed-borne infection and 78 % germination in 2000-2001. Prevention of seedling infection from soil-borne inoculum by the seed treatments, which would have increased importance in later drilled trials where seedling emergence was slower, is probably responsible for increased emergence and establishment above untreated seedlots. Emergence from the untreated seedlots was so severely reduced, subsequent compensatory tillering was not sufficient to avoid yield losses.

FURTHER WORK

This work has shed some light on the epidemiology of *Fusarium* seedling blight caused by *M. nivale*, although many questions remain unanswered. These questions could be addressed by investigating the following areas:

(i) Further controlled environment studies to characterise the effects of cultivar and seedlot vigour on the *M. nivale*-host interaction.

(ii) *In vitro* studies to investigate the site and depth of *M. nivale* infection in seeds and its role in seedling blight severity and chemical control under different environmental conditions.

(iii) Light and electron microscopy investigations to elucidate the transfer of *M. nivale* from seed-borne infection to seedlings.

(iv) Additional field and controlled environment investigations into specific temperature and soil water content periods affecting seedling emergence from *M. nivale* infected and pathogen-free seedlots.

(v) Determine the influence of seedbed conditions (drilling depth, cultivation techniques, soil type, particle size and nutrient status) on seedling emergence and *M. nivale* seedling blight severity.

(vi) Further investigations into the effectiveness of seed treatments at controlling seedling blight under reduced seed rates and minimum tillage systems.

(vii) Effects of seed treatments on pathogen-free seed in harsh seedbed conditions.

CHAPTER 10

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CHAPTER 11

Appendices

APPENDIX A

DNA extraction mixtures and PCR reaction ingredients.

CTAB buffer

sorbitol 23 g; N-lauryl sarcosine 10 g; CTAB 8 g; EDTA 8 g; sodium chloride 87.7 g; polyvinylpolypyrrolidone 10 g; and water to 1 litre.

TE buffer

10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

TAE buffer

40 mM Tris-acetate, 1 mM EDTA, pH 8.0.

50 µl PCR reaction mixtures

100 µM concentration of each nucleotide

20 U of Red Hot *Taq* polymerase (Abgene, Epson, UK) per ml

100 nM SEMF (CGTACGGTTGGATGCCGAG) and SEMR (GTCCTCAGTCCCAGCGGC)

10 mM Tris-HCl (pH 8.3)

1.5 mM $MgCl_2$

50 mM KCl

100 µg of gelatin per ml

10 µl DNA sample

10 µl internal standard

Reaction mixture was overlaid with mineral oil.

APPENDIX B

Median air temperature in an unheated polytunnel during an investigation into the effect of delayed imbibition on subsequent seedling growth for *Microdochium nivale* infected and pathogen-free winter wheat seedlots (cv. Equinox; seedlots four and six).